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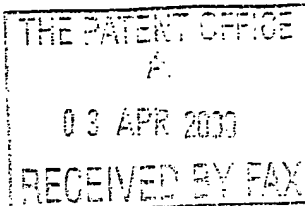


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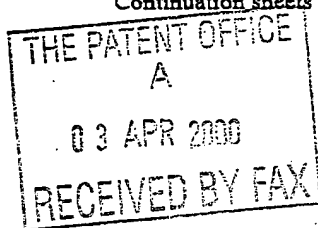
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2. Patent application number (The Patent Office will fill in this part)	0008049.9		
3. Full name, address and postcode of the or of each applicant (underline all surnames)	Antisoma Research Limited West Africa House Hanger Lane Ealing London W5 3QR United Kingdom		
Patents ADP number (if you know it)	7869100001		
If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom		
4. Title of the invention	COMPOUNDS FOR TARGETING		
5. Name of your agent (if you have one)	ERIC POTTER CLARKSON		
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DUPLICATE

COMPOUNDS FOR TARGETING

The present invention relates to cytotoxic compounds that have a high
5 avidity for, and can be targeted to, selected cells. Specifically, the
invention provides compounds comprising a cytotoxic portion having
DNA endonucleolytic activity and a target-cell specific portion having
specificity for human polymorphic epithelial mucin (PEM).

10 Background

The cell-specific targeting of compounds that are directly, or indirectly,
cytotoxic has been proposed as a way to combat diseases such as cancer.
Bagshawe and his co-workers have disclosed (Bagshawe (1987) *Br. J.*
15 *Cancer* 56, 531; Bagshawe *et al* (1988) *Br. J. Cancer* 58, 700; WO
88/07378) conjugated compounds comprising an antibody or part thereof
and an enzyme, the antibody being specific to tumour cell antigens and the
enzyme acting to convert an innocuous pro-drug into a cytotoxic
compound. The cytotoxic compounds were alkylating agents, *e.g.* a
20 benzoic acid mustard released from *para*-N-bis(2-
chloroethyl)aminobenzoyl glutamic acid by the action of *Pseudomonas sp.*
CPG2 enzyme.

An alternative system using different pro-drugs has been disclosed
25 (WO 91/11201) by Epenetos and co-workers. The cytotoxic compounds
were cyanogenic monosaccharides or disaccharides, such as the plant
compound amygdalin, which release cyanide upon the action of a β -
glucosidase and hydroxynitrile lyase.

In a further alternative system, the use of antibody-enzyme conjugates containing the enzyme alkaline phosphatase in conjunction with the pro-drug etoposide 4'-phosphate or 7-(2'-aminoethyl phosphate)mitomycin or
5 a combination thereof have been disclosed (EP 0 302 473; Senter *et al* (1988) *Proc. Natl. Acad. Sci. USA* 85, 4842).

Rybak and co-workers have disclosed (Rybak *et al* (1991) *J. Biol. Chem.* 266, 21202; WO 91/16069) the cytotoxic potential of a monomeric
10 pancreatic ribonuclease when injected directly into *Xenopus* oocytes and the cytotoxic potential of monomeric RNase coupled to human transferrin or antibodies directed against the transferrin receptor. The monomeric RNase hybrid proteins were cytotoxic to human erythroleukaemia cells *in vitro*.

15 Other approaches are the *in vivo* application of streptavidin conjugated antibodies followed, after an appropriate period, by radioactive biotin (Hnatowich *et al* (1988) *J. Nucl. Med.* 29, 1428-1434), or injection of a biotinylated mAb followed by radioactive streptavidin (Paganelli *et al*
20 (1990) *Int. J. Cancer* 45, 1184-1189). A pilot radioimmunolocalisation study in non-small cell lung carcinomas was conducted with encouraging results (Kalofonos *et al* (1990) *J. Nucl. Med.* 31, 1791-1796).

Apart from these examples, it is rather more common to see biotinylated
25 antibodies and streptavidin-enzyme conjugates, which are used in enzyme-linked immunosorbent assays.

These previous systems have used relatively large antibody-enzyme,

antibody-streptavidin or antibody-biotin conjugates and may comprise portions of non-mammalian origin which are highly immunoreactive.

We have now devised improved compounds for targeting cells to be
5 destroyed.

Summary of Invention

A first aspect of the invention provides a compound comprising a target
10 cell-specific portion and a cytotoxic portion characterised in that the target cell-specific portion comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof, and the cytotoxic portion has endonucleolytic activity.

15 By "target cell specific" portion we mean the portion of the compound which comprises one or more binding sites which recognise and bind to polymorphic epithelial mucin (PEM) on the target cell. Upon contact with the target cell, the target cell specific portion is preferably internalised along with the cytotoxic portion. Such internalisation results in the
20 cytotoxic portion being delivered to the cell cytosol, where it has access to the cell's nucleic acid molecules.

The target cell-specific portion of the compounds of the invention comprises an humanised monoclonal antibody having specificity for
25 polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof.

Polymorphic epithelial mucin, or PEM, is a component of the human milk

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fat globule. PEM is expressed by cells in several body tissues and is also found in urine. Significantly, PEM is known to be expressed in epithelial cancer cells, notably in ovarian, gastric, colorectal and pancreatic cancer cells.

5

Monoclonal antibodies which will bind to PEM are already known, but in any case, with today's techniques in relation to monoclonal antibody technology, antibodies can be prepared to most antigens. The antigen-specific portion may be a whole antibody, a part of an antibody (for example a Fab or F(ab')₂ fragment), a synthetic antibody fragment (for example a single chain Fv fragment [ScFv]), or a peptide/peptidomimetic or similar. Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in *"Monoclonal Antibodies: A manual of techniques"*, H Zola (CRC Press, 1988) and in *"Monoclonal Hybridoma Antibodies: Techniques and Applications"*, J G R Hurrell (CRC Press, 1982) and *Antibody Engineering, A Practical Approach*, McCafferty, J. et al, ed. (IRL Pres, 1996).

20 By 'humanised monoclonal antibody' we include monoclonal antibodies having at least one chain wherein the framework regions are predominantly derived from a first, acceptor monoclonal antibody of human origin and at least one complementarity-determining region (CDR) is derived from a second, donor monoclonal antibody having specificity
25 for PEM. The donor monoclonal antibody may be of human or non-human origin, for example it may be a murine monoclonal antibody.

Preferably, both chains of the humanised monoclonal antibody comprise

5

CDRs grafted from a donor monoclonal antibody having specificity for PEM.

Advantageously, the CDR-grafted (*i.e.* humanised) chain comprises two
5 or all three CDRs derived from a donor antibody having specificity for PEM.

Conveniently, the humanised monoclonal antibody comprises only human
framework residues and CDRs from a donor antibody having specificity
10 for PEM.

However, it will be appreciated by those skilled in the art that in order to
maintain and optimise the specificity of the humanised antibody it may be
necessary to alter one or more residues in the framework regions such that
15 they correspond to equivalent residues in the donor antibody.

Conveniently, the framework regions of the humanised antibody are
derived from an human IgG monoclonal antibody.

20 Methods of making humanised monoclonal antibodies are well-known in
the art, for example see Jones *et al.* (1986) *Nature* 321:522-525,
Riechmann *et al.* (1988) *Nature* 332:323-327, Verhoeyen *et al.* (1988)
Science 239:1534-1536 and EP 239 400 (to Winter).

25 In a preferred embodiment of the first aspect of the invention, the target
cell-specific portion comprises an humanised HMFG-1 monoclonal
antibody or an antigen binding fragment thereof.

6

HMFG antibodies are raised against human milk fat globule (HMFG), in a delipidated state (see Taylor-Papadimitriou *et al.*, 1981, *Int. J. Cancer* 28:17-21 and Gendler *et al.*, 1988, *J. Biol. Chem.* 236:1282-12823).

HMFG-1 monoclonal antibodies bind to a particular component of
5 HMFG, namely polymorphic epithelial mucin (PEM). Binding is thought to involve the amino acid sequence APDTR within the twenty amino acid tandem repeats of the *muc-1* gene product.

Exemplary humanised HMFG-1 antibodies are disclosed in WO 92/04380.
10

Advantageously, the target cell-specific portion is an humanised HMFG-1 monoclonal antibody.

In a preferred embodiment of the first aspect of the invention, the target
15 cell-specific portion comprises a fragment of an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), said fragment retaining the antigen binding properties of the parent antibody.

The variable heavy (V_H) and variable light (V_L) domains of the antibody
20 are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further confirmation was found by "humanisation" of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent parented antibody
25 (Morrison *et al* (1984) *Proc. Natl. Acad. Sci. USA* 81, 6851-6855).

That antigenic specificity is conferred by variable domains and is independent of the constant domains is known from experiments involving

the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better *et al* (1988) *Science* 240, 1041); Fv molecules (Skerra *et al* (1988) *Science* 240, 1038); disulphide-linked Fv molecules (Young *et al.*, 1995, *FEBS Lett.* 377:135-139); single-chain Fv (ScFv) molecules where the V_H and V_L partner domains are linked via a flexible oligopeptide (Bird *et al* (1988) *Science* 242, 423; Huston *et al* (1988) *Proc. Natl. Acad. Sci. USA* 85, 5879) and single domain antibodies (dAbs) comprising isolated V domains (Ward *et al* (1989) *Nature* 341, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) *Nature* 349, 293-299.

By "ScFv molecules" we mean molecules wherein the V_H and V_L partner domains are linked via a flexible oligopeptide.

Chimaeric antibodies are discussed by Neuberger *et al* (1988, 8th *International Biotechnology Symposium* Part 2, 792-799).

The advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments allows for rapid clearance, and may lead to improved tumour to non-tumour ratios. Fab, Fv, ScFv, disulphide Fv and dAb antibody fragments can all be expressed in and secreted from bacteria, such as *E. coli*, or eukaryotic expression systems such as Yeast or mammalian systems, thus allowing the facile production of large amounts of the said fragments.

Whole antibodies, and F(ab')₂ fragments are "bivalent". By "bivalent" we

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mean that the said antibodies and $F(ab')_2$ fragments have two antigen combining sites. In contrast, Fab, Fv, ScFv, disulphide Fv and dAb fragments are monovalent, having only one antigen combining site.

5 Preferably, the target cell-specific portion of the compounds of the invention comprises an antigen binding fragment of the humanised antibody selected from the group consisting of Fab-like molecules, such as Fab and $F(ab')_2$, Fv molecules, disulphide-linked Fv molecules, ScFv molecules and single domain antibodies (dAbs).

10

More preferably, the target cell-specific portion comprises a Fab molecule or a $F(ab')_2$ molecule.

15 Yet more preferably, the target cell-specific portion comprises at least a part of one or both of the amino acid sequences shown in Figure 3.

Most preferably, the target cell-specific portion comprises both of the amino acid sequences shown in Figure 3.

20 Preferably, the target cell-specific portion recognises the target cell with high avidity.

By "high avidity" we mean that the target cell-specific portion recognises the target cell with a binding constant of at least $K_d = 10^{-6}$ M, preferably
25 at least $K_d = 10^{-9}$ M, suitably $K_d = 10^{-10}$ M, more suitably $K_d = 10^{-11}$ M, yet more suitably still $K_d = 10^{-12}$ M, and more preferably $K_d = 10^{-15}$ M or even $K_d = 10^{-18}$ M.

Preferably, the target cell-specific portion comprises an antigen binding fragment of an humanised HMFG-1 monoclonal antibody, *e.g.* an Fab or F(ab')₂ fragment thereof, wherein a hinge region contains a mutation (*i.e.* wherein the hinge is a variant or hybrid of a naturally occurring hinge). More preferably, the variant hinge comprises the amino acid sequence CCVECPPCPAPE.

By 'cytotoxic portion' we mean a portion having endonucleolytic activity which is toxic to the cell if it is to reach, and preferably enter said cell.

10

In a preferred embodiment of the first aspect of the invention, the cytotoxic portion has DNA endonucleolytic activity.

Advantageously, the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease.

Examples of known DNA endonucleases include bovine DNase I (see Worrall and Conolly, 1990, *J. Biol. Chem.* 265:21889-21895). Human pancreatic DNase I has also been cloned (see Shak *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* 87:9188-9192 and Hubbard *et al.*, 1992, *New Eng. J. Med.* 326:812-815).

Preferably, the endonuclease is a mammalian deoxyribonuclease I.

More preferably, the endonuclease is a human deoxyribonuclease I.

Most preferably, the cytotoxic portion comprises the amino acid sequence shown in Figure 2.

Preferably, the cytotoxic portion of the compound of the invention is capable of oligomerisation, *e.g.* dimerisation. Attachment of the target-cell specific portion to a cytotoxic portion capable of oligomerisation provides a method for increasing the number of binding sites to the target cell. For example, if the target cell-specific portion is joined to a portion capable of forming a dimer then the number of target cell-specific binding sites is two; if the target cell-specific portion is joined to a portion capable of forming a tetramer then the number of target cell-specific binding sites is four. The number of target cell-specific binding sites is greater than one and the compounds may therefore have a greater avidity for the target cell than do compounds which only have one target cell-specific binding site.

It is preferable for the cytotoxic portion of the compound of the invention capable of oligomerisation to contain no interchain disulphide bonds nor intrachain disulphide bonds; to be well characterised; to be non-toxic; to be stable; to be amenable to preparation in a form suitable for pre-clinical or clinical use or be in pre-clinical or clinical use; and for the subunit monomers to have a high affinity for each other, that is they contain one or more subunit binding sites.

Advantageously, the cytotoxic portion is of mammalian, preferably human, origin. The use of the said mammalian proteins as the cytotoxic portion of the compound of the invention is advantageous since such compounds are less likely to give rise to undesirable immune reactions.

It will be appreciated by those skilled in the art that the cytotoxic portion

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may be a variant of a naturally occurring endonuclease.

By "a variant" we include cytotoxic portions comprising of a naturally occurring endonuclease wherein there have been amino acid insertions, deletions or substitutions, either conservative or non-conservative, such that the changes do not substantially reduce the endonuclease activity of the variant compared to that of the naturally occurring endonuclease. For example, the variant may have increased activity compared to the naturally occurring endonuclease

10

Such variants may be made using methods of protein engineering and site-directed mutagenesis commonly known in the art (for example, see Sambrook *et al.*, 1989, *Molecular cloning: A Laboratory Manual*. 2nd edition, Cold Spring Harbor Laboratory Press, NY, USA).

15

In an alternative embodiment, the endonuclease is a restriction endonuclease, such as a microbial type II restriction endonuclease. Exemplary type II restriction endonucleases include *Bam*HI, *Hind*III, *Msp*I, *Sau*3AI, *Hin*fI, *Not*I and *Eco*RI.

20

In another preferred embodiment of the first aspect of the invention, a nuclear localization signal is incorporated into the compound.

Preferably, the nuclear localization signal comprises a nuclear localization signal from the SV40 large T antigen (Kalderon *et al.*, 1984, *Cell* 39:499-509), and specifically the amino acid sequence PKKKRKV. Inclusion of a nuclear localization signal encourages the compound of the invention to gain access to the chromosomal DNA during the periods of the cell cycle

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when the nuclear membrane is intact, since the nuclear pores are permeable to large molecules incorporating said nuclear localization signal.

- 5 In a further preferred embodiment of the first aspect of the invention, the target cell-specific portion and the cytotoxic portion are fused to create a fusion compound.

- By "fusion compound" we include a compound comprising one or more
10 functionally distinct portions, wherein the distinct portions are contained within a single polypeptide chain produced by recombinant DNA techniques.

- Preferably, the target-cell specific and the cytotoxic portion of the fusion
15 compound of the invention separated by a linker sequence, for example to allow greater flexibility of the portions relative to one another.

More preferably, the linker sequence comprises a GG dipeptide.

- 20 Most preferably the linker sequence is or comprises GG or GSGG.

- Alternatively, the target-cell specific and the cytotoxic portion of the compound of the invention are separate moieties linked together by any of the conventional ways of cross-linking polypeptides, such as those
25 generally described in O'Sullivan *et al Anal. Biochem.* (1979) 100, 100-108. For example, the antibody portion may be enriched with thiol groups and the enzyme portion reacted with a bifunctional agent capable of reacting with those thiol groups, for example the N-hydroxysuccinimide

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ester of iodoacetic acid (NHIA) or N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP). Amide and thioether bonds, for example achieved with m-maleimidobenzoyl-N-hydroxysuccinimide ester, are generally more stable *in vivo* than disulphide bonds.

5

A second aspect of the invention provides a nucleic acid encoding a compound according to the first aspect of the invention, or a target cell-specific portion or cytotoxic portion thereof.

- 10 By "nucleic acid molecule" we include DNA, cDNA and mRNA molecules.

A further aspect of the present invention provides a method of making a compound according to the first aspect of the invention, said method
15 comprising expressing one or more nucleic acid molecules according to the second aspect of the invention in a host cell and isolating the compound therefrom.

It is preferable that the two portions of the compound of the invention are
20 produced as a fusion compound by recombinant DNA techniques, whereby a length of DNA comprises respective regions encoding the two portions of the compound of the invention either adjacent one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the compound. The benefits in making the
25 compound of the invention using recombinant DNA techniques are several fold. Firstly, it enables a high degree of precision with which the two portions of the compound can be joined together. Secondly, the construction of compounds which are "hetero-oligomeric" can be

controlled by the expression of the different recombinant DNA molecules encoding each of the different type of subunit of the "hetero-oligomer" in the same host cell.

- 5 By "hetero-oligomer" we mean those compounds in which two or more different cell-specific portions are joined to either the same or to different subunits which are capable of oligomerisation. The expression, in the same host cell of two compounds, of A and B, each with different target cell specific portions but with a common second portion capable of
- 10 oligomerisation will result in a mixed population of compounds. For example, if the common second portion is capable of dimerisation, three potential compounds will be produced: A_2 , AB and B_2 , in a ratio of 1:2:1, respectively.
- 15 The separation of the desired compound with each of the different cell specific portions, that is AB, can be achieved by two step affinity chromatography.
- 20 Application of the mixture of compounds to an affinity column specific for A will result in the binding of A_2 and AB. These compounds are eluted from this first column, and then applied to an affinity column specific for B. This will result in AB, but not A_2 , being bound to the column. Finally, the desired product AB, can be eluted.
- 25 Of course, the order in which the affinity columns are used is not important.

The same principle of separating those compounds with two or more

15

different binding sites can be applied to the purification of the desired compounds from mixtures of other hetero-oligomers.

Conceivably, the two portions of the compound may overlap wholly or
5 partly.

The nucleic acid is then expressed in a suitable host to produce a polypeptide comprising the compound of the invention. Thus, the nucleic acid encoding the compound of the invention or a portion thereof may be
10 used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the polypeptide of the invention. Such techniques include those disclosed in US Patent Nos. 4,440,859 issued 3 April 1984 to Rutter
15 *et al*, 4,530,901 issued 23 July 1985 to Weissman, 4,582,800 issued 15 April 1986 to Crowl, 4,677,063 issued 30 June 1987 to Mark *et al*, 4,678,751 issued 7 July 1987 to Goeddel, 4,704,362 issued 3 November 1987 to Itakura *et al*, 4,710,463 issued 1 December 1987 to Murray, 4,757,006 issued 12 July 1988 to Toole, Jr. *et al*, 4,766,075 issued 23
20 August 1988 to Goeddel *et al* and 4,810,648 issued 7 March 1989 to Stalker, all of which are incorporated herein by reference.

The nucleic acid encoding the compound of the invention or a portion thereof may be joined to a wide variety of other nucleic acid sequences for
25 introduction into an appropriate host. The companion nucleic acid will depend upon the nature of the host, the manner of the introduction of the nucleic acid into the host, and whether episomal maintenance or integration is desired.

16

It will be appreciated that in order to prevent expression of the cytotoxic portion of the compound of the invention from killing the host cells in which it is expressed, it may be necessary to link the nucleic acid of the
5 second aspect of the invention to a signal sequence capable of directing secretion of the expressed compound (or portion) out of the host cell. Signal sequences will be selected according to the type of host cell used. Exemplary signal sequences include the *ompA* signal sequence (for example, see Takahara *et al.*, 1985, *J. Biol. Chem.* 260(5):2670-2674).

10

Generally, the nucleic acid is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the nucleic acid may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences
15 recognised by the desired host, although such controls are generally available in the expression vector. The vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed by the vector. Therefore, it will be necessary to select for transformed host cells. One selection technique involves incorporating
20 into the expression vector a nucleic acid sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance. Alternatively, the gene for such selectable trait can be on another vector, which is used to co-transform the desired host cell.

25

Host cells that have been transformed by the recombinant nucleic acid of the invention are then cultured for a sufficient time and under appropriate conditions known to those skilled in the art in view of the teachings

disclosed herein to permit the expression of the polypeptide, which can then be recovered.

Many expression systems are known, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae* and *Pichia pastoris*), filamentous fungi (for example *Aspergillus*), plant cells, animal cells (for example COS-1, COS-7, CHO, NIH 3T3, NS0 and BHK cells) and insect cells (for example *Drosophila*, SF9 cells).

Those vectors that include a replicon such as a procaryotic replicon can also include an appropriate promoter such as a procaryotic promoter capable of directing the expression (transcription and translation) of the genes in a bacterial host cell, such as *E. coli*, transformed therewith.

A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with exemplary bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention.

Typical procaryotic vector plasmids are pUC18, pUC19, pBR322 and pBR329 (available from Biorad Laboratories, Richmond, CA, USA), pTrc99A and pKK223-3 (available from Pharmacia Piscataway, NJ, USA) and the pET system (T7 promoter, Novagen Ltd).

A typical mammalian cell vector plasmid is pSVL available from Pharmacia, Piscataway, NJ, USA. This vector uses the SV40 late promoter to drive expression of cloned genes, the highest level of

expression being found in T antigen-producing cells, such as COS-1 cells.

An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia. This vector uses the glucocorticoid-inducible
5 promoter of the mouse mammary tumour virus long terminal repeat to drive expression of the cloned gene.

Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037,
10 USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers *his3*, *trp1*, *leu2* and *ura3*. Plasmids pRS413-416 are Yeast Centromere plasmids (YCps).

15 Further useful vectors for transformation of yeast cells, such as *Pichia*, include the 2 μ plasmid pYX243 (available from R and D Systems Limited) and the integrating vector pPICZ series (available from Invitrogen).

A variety of methods have been developed to operatively link DNA to
20 vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

25

Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion as described

earlier, is treated with bacteriophage T4 DNA polymerase or *E. coli* DNA polymerase I, enzymes that remove protruding, 3'-single-stranded termini with their 3'-5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

5

The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as
10 bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

15

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CN, USA.

20 A desirable way to modify the nucleic acid encoding the compound of the invention or a portion thereof is to use the polymerase chain reaction as disclosed by Saiki *et al* (1988) *Science* 239, 487-491.

In this method the nucleic acid to be enzymatically amplified is flanked by
25 two specific oligonucleotide primers which themselves become incorporated into the amplified nucleic acid. The said specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

Exemplary genera of yeast contemplated to be useful in the practice of the present invention are *Pichia*, *Saccharomyces*, *Kluyveromyces*, *Candida*, *Torulopsis*, *Hansenula*, *Schizosaccharomyces*, *Citeromyces*, *Pachysolen*,
5 *Debaromyces*, *Metschnikowia*, *Rhodospiridium*, *Leucosporidium*,
Botryoascus, *Sporidiobolus*, *Endomycopsis*, and the like. Preferred genera are those selected from the group consisting of *Pichia*, *Saccharomyces*, *Kluyveromyces*, *Yarrowia* and *Hansenula*. Examples of *Saccharomyces* are *Saccharomyces cerevisiae*, *Saccharomyces italicus* and
10 *Saccharomyces rouxii*. Examples of *Kluyveromyces* are *Kluyveromyces fragilis* and *Kluyveromyces lactis*. Examples of *Hansenula* are *Hansenula polymorpha*, *Hansenula anomala* and *Hansenula capsulata*. *Yarrowia lipolytica* is an example of a suitable *Yarrowia* species.

15 Methods for the transformation of *S. cerevisiae* are taught generally in EP 251 744, EP 258 067 and WO 90/01063, all of which are incorporated herein by reference.

Suitable promoters for *S. cerevisiae* include those associated with the
20 *PGK1* gene, *GAL1* or *GAL10* genes, *CYC1*, *PHO5*, *TRP1*, *ADH1*, *ADH2*, the genes for glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, triose phosphate isomerase, phosphoglucose isomerase, glucokinase, α -mating factor pheromone, α -mating factor pheromone, the *PRB1* promoter, the *GUT2* promoter, and
25 hybrid promoters involving hybrids of parts of 5' regulatory regions with parts of 5' regulatory regions of other promoters or with upstream activation sites (eg the promoter of EP-A-258 067).

The transcription termination signal is preferably the 3' flanking sequence of a eukaryotic gene which contains proper signals for transcription termination and polyadenylation. Suitable 3' flanking sequences may, for example, be those of the gene naturally linked to the expression control
5 sequence used, i.e. may correspond to the promoter. Alternatively, they may be different in which case the termination signal of the *S. cerevisiae* *AHD1* gene is preferred.

The present invention also relates to a host cell transformed with a
10 polynucleotide vector construct of the present invention. The host cell can be either procaryotic or eukaryotic. Bacterial cells are preferred procaryotic host cells and typically are a strain of *E. coli* such as, for example, the *E. coli* strains DH5 available from Bethesda Research Laboratories Inc., Bethesda, MD, USA, and RR1 available from the
15 American Type Culture Collection (ATCC) of Rockville, MD, USA (No ATCC 31343). Preferred eukaryotic host cells include yeast and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic cell line. Preferred eukaryotic host cells include Chinese hamster ovary (CHO) cells available from the ATCC
20 as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658 and monkey kidney-derived COS-1 cells available from the ATCC as CRL 1650 or WSØ cells.

Transformation of appropriate cell hosts with a nucleic acid constructs of
25 the present invention is accomplished by well known methods that typically depend on the type of vector used. With regard to transformation of procaryotic host cells, see, for example, Cohen *et al.* *Proc. Natl. Acad. Sci. USA*, 69: 2110 (1972); and Sambrook *et al.*

Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989). Transformation of yeast cells is described in Sherman *et al*, *Methods In Yeast Genetics, A Laboratory Manual*, Cold Spring Harbor, NY (1986). The method of
5 Beggs. *Nature*, 275: 104-109 (1978) is also useful. With regard to vertebrate cells, reagents useful in transfecting such cells, for example calcium phosphate and DEAE-dextran or liposome formulations, are available from Stratagene Cloning Systems, or Life Technologies Inc, Gaithersburg, MD 20877, USA.

10

Successfully transformed cells, *i.e.* cells that contain a nucleic acid construct of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an expression construct of the present invention can be grown to produce the
15 polypeptide of the invention. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern. *J. Mol. Biol.*, 98: 503 (1975) or Berent *et al*, *Biotech.*, 3: 208 (1985). Alternatively, the presence of the protein in the supernatant can be detected using antibodies as described below.

20

In addition to directly assaying for the presence of recombinant nucleic acid, successful transformation can be confirmed by well known immunological methods when the recombinant nucleic acid is capable of directing the expression of the protein. For example, cells successfully
25 transformed with an expression vector produce proteins displaying appropriate antigenicity. Samples of cells suspected of being transformed are harvested and assayed for the protein using suitable antibodies.

Thus, in addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium. Preferably, the culture also
5 contains the protein.

Nutrient media useful for culturing transformed host cells are well known in the art and can be obtained from several commercial sources.

10 A third aspect of the invention provides a vector comprising a nucleic acid according to the second aspect of the invention.

A fourth aspect of the invention provides a host cell comprising a vector according to the third aspect of the invention.

15

Preferably, the host cell is a mammalian cell.

More preferably the host cell is NS0.

20 A fifth aspect of the invention provides a pharmaceutical composition comprising a compound according to the first aspect of the invention and a pharmaceutically acceptable carrier.

The compounds and compositions of the invention are administered in any
25 suitable way, usually parenterally, for example intravenously, intraperitoneally or, preferably (for bladder cancer), intravesically (i.e. into the bladder), in standard sterile, non-pyrogenic formulations of diluents and carriers, for example isotonic saline (when administered

24

intravenously).

A sixth aspect of the invention provides a compound according to the first aspect of the invention for use in medicine.

5

The compounds and compositions of the invention may be used to treat a patient with any disease involving a dysfunction of a population of cells expressing PEM, said compounds and compositions selectively targeting and destroying said population of cells within a patient. For example, said
10 compounds and compositions may be used in the treatment of cancer, *e.g.* cancer of the breast, ovaries, lung, stomach, intestines, blood *etc.* Thus, anti-tumour cell antigen antibodies can be used to deliver a cytotoxic portion with endonuclease activity to a tumour cell. Antibodies that are internalised upon contact with the target antigen are used, such that the
15 cytotoxic portion enters the cytosol of the tumour cell, where it can trigger cell death.

In principle, the compounds and compositions of the invention may be used to treat any mammal, including pets such as dogs and cats and
20 agriculturally important animals such as cows, horses, sheep and pigs.

Preferably, the patient is human.

A seventh aspect of the invention provides the use of a compound
25 according to first aspect of the invention in the preparation of a medicament for treating a mammal having said target cells to be destroyed.

25

Preferably, the medicament is for treating cancer, such as ovarian cancer.

A eighth aspect of the invention provides a method of treating a mammal having target cells to be destroyed, the method comprising administering
5 a compound according to the first aspect of the invention to said mammal.

In a preferred embodiment of the seventh and eighth aspects of the invention, the mammal is a human.

10 Preferably, the target cells to be destroyed are cancer cells. More preferably, the cancer cells are epithelial cancer cells, such as ovarian, gastric, colorectal and/or pancreatic cancer cells. Most preferably, the cancer cells are ovarian cancer cells.

15 The invention will now be described in detail with reference to the following figures and examples:

Figure 1 shows the complete coding sequence of human DNase I.

20 Figure 2 shows the DNase I sequence used in the exemplary constructs (*e.g.* pAS41).

Figure 3 shows the HMFG1 light chain insert (A) and heavy chain insert (B) used in the exemplary constructs (*e.g.* pAS6).

25

Figure 4 shows the linker and hinge-linker oligonucleotides used in (A) the whole antibody-DNase and (B) the Fab'2-DNase exemplary constructs. Note, in Figure 4(A) a deletion of one or more codons

between the HMFG1 hinge and the linker is represented as ΔG .

Figure 5 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS23 (linker sequence underlined).

5

Figure 6 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS27 (linker sequence and NLS underlined).

10 Figure 7 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS34 (linker sequence underlined).

Figure 8 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS35 (linker sequence underlined).

15 The lower case 'g' represents a silent mutation caused by PCR amplification.

Figure 9 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS36 (linker sequence underlined).

20 The lower case 'c' represents a silent mutation caused by PCR amplification.

Figure 10 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS37 (linker sequence and NLS underlined).

25

Figure 11 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS38 (linker sequence and NLS

underlined). The lower case 'g' represents a silent mutation caused by PCR amplification.

Figure 12 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS39 (linker sequence underlined). The lower case 'c' represents a silent mutation caused by PCR amplification.

Figure 13 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS101 (linker sequence underlined).

Figure 14 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS102 (hybrid hinge and linker sequence underlined).

Figure 15 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS103 (hybrid hinge and linker sequence underlined).

Figure 16 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS104 (hybrid hinge and linker sequence underlined, mutations (compared to pAS103) at positions 775 and 924 are shaded).

Figure 17 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS105 (linker sequence and NLS underlined).

Figure 18 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS106 (hybrid hinge + linker sequence and NLS are underlined).

5

Figure 19 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS107 (hybrid hinge + linker sequence and NLS are underlined).

- 10 Figure 20 shows a schematic diagram of the pEE6 expression vector used in the exemplary constructs.

Figure 21 shows autoradiographs from immuno-precipitation experiments with metabolically labelled transient transfectants:

15

GEL A

Lane 1 shows the precipitation of supernatant from mock-transfected cells.

- 20 Lane 2 is from cells transfected with hHMFG-1 (construct 6) giving expected molecular weights of about 51.2 and 26.4 kDa for the heavy and light chains, respectively.

- 25 Lane 3 shows construct 34 antibody construct which has human DNase I fused to the C-terminus of the heavy chain gene. As expected, the size of the heavy chain gene has increased to about 80.7 kDa.

Samples from whole antibody DNase I constructs 35, 36 and 39 were run on the gel (Lanes 4 to 6) but were not sufficiently well expressed to be visible, in this experiment.

29

In subsequent experiments using this method, construct 39 was detectable but weak, and constructs 35 and 36 were detectable but very weak. Constructs 37 and 38 have not been tested in this assay system.

5 Lanes 8 to 10 are fusion of humanised HMFG1 F(ab')₂ with human DNase I (constructs 41, 23 and 102, respectively). F(ab')₂ alone was included in this set of experiments (lane 7, construct 41) but did not express, this was included in later experiments (see gels C and D).

10 In addition to the light chain (about 26.4 kDa) and the Fd-DNase I fusion (about 56.6 kDa), a third major band is observed at around 40 kDa. Interestingly, this band is observed in the humanised HMFG-1 fusions but not in the antibody alone. Since an anti-F(ab')₂ antibody was used for immuno-precipitation, it is unlikely that this can be proteolysis between immunoglobulin and DNase I sequence.

15 It probably represents a population of polypeptide produced by premature transcriptional termination (due to DNase I sequence in the 3'-end of the fusion mRNA).

GEL B

20 This is the non-reducing gel counterpart to gel A, described above.

Lane 1 is the mock-transfected control cells and lanes 2 and 3 are from the cells transfected with humanised HMFG1 alone (construct 6) and the humanised HMFG-1 fused at the C-terminus to human DNase I, respectively. As before, lanes 4 to 6 are from cell supernatants from cells transfected with constructs 35, 36 and 39.

25 The gel shows that both the whole antibody and the antibody-DNase I fusion are assembled, with the DNase fusion giving a higher molecular weight compared to the antibody alone.

Figure 22 shows a typical standard curve used to determine the concentration of PDTRP-binding material in the supernatants of transiently transfected L761h cells. Each point on the curve has been determined
5 twice.

Figure 23 shows typical standard curves used to determine the concentration of bovine DNase I.

10 Figure 24 shows corrected DNase I activity in transiently expressed humanised HMFG1 whole antibody-human DNase I fusions (*i.e.* pAS34, pAS34, pAS35 and pAS6[control]).

Figure 25 shows the corrected DNase I activity in transiently expressed
15 humanised HMFG1 F(ab')₂-human DNase I fusions (*i.e.* pAS101, pAS102, pAS103 and pAS41[control]).

Figure 26 shows results of the cytotoxicity assay.

20 Figure 27 shows the % of MCF7 cells killed after incubation with the exemplary constructs.

EXAMPLES

(A) Mammalian expression of humanised HMFG1-DNase constructs

5 The human HMFG1 light and heavy chain (with or without engineering a fusion to human DNase I), were cloned into the pEE6 expression vector system for expression in mammalian CHO or myeloid NS0 cells (see figure 20). The vector system was originally developed by Celltech Ltd (UK) and is now owned by al-Lonza (see Young & Owens, 1994, *J.*
10 *Immunol. Meth.* 168:149-165). The vector consists of two human cytomegalovirus promoters (hCMV) for both the heavy and light chain genes. Each transcription unit is completed by the poly-adenylation signal (pA) with an optional immunoglobulin terminator sequence (Ig term.) located between the heavy and light chain transcription units. Propagation
15 in *E.coli* can be selected for by the presence on an ampicillin resistance gene (not shown in Fig 20). The inclusion of a glutamine synthetase gene (GS) in the vector allows the stable NS0 transfectomas to be selected by growth in glutamine free media, since NS0 cells are GS⁻ and cannot otherwise grow in glutamine free media.

20

Exemplary humanized HMFG1-DNase I fusion constructs of the invention are detailed in figures 5 to 19.

(B) Immuno-precipitation of metabolically labelled transient 25 transfectants

CHO-L761h cells (Cockett *et al.*, 1990, *Nuc. Acids Res.* 19:319-325) were transfected, according to the modification of Gorman *et al.*, 1985),

with expression vectors containing either whole HMFG1 antibody or F(ab')₂ fragment of the antibody along with the various fusion constructs of their respective heavy chains and human DNase I. The cells were then incubated with either 50 μ Ci ³⁵S methione for 72 h in methionine-free medium. Secreted product was precipitated with a rabbit anti-human F(ab')₂ antibody bound to protein A Sepharose. Bound material was eluted in either reducing or non-reducing SDS-PAGE loading buffer and run on gels. The autoradiographs (see Figure 21) above were generated from those gels after drying them.

10

(C) Estimation of the efficiency of DNase constructs in supernatants

Introduction

15 This set of experiments was designed to standardise the amount of construct in a given DNase I activity assay and to allow us to comment on the amount of activity a particular construct possesses. Given that the antibody-DNase I fusions are so different to the F(ab')₂-DNase I fusions it is best not to compare the two groups. Once we have purified the protein, we will have a better idea of the exact molecular configuration of all species. Then, and only then, will it be sensible to compare amongst groups.

20

Determination of concentration of constructs

25

The concentration of constructs in supernatants from transiently transfected L761H cells was determined in a PDTRP-binding ELISA. To each well of a Maxisorb 96-well ELISA plate (Nunc) was added 100 μ l of

carbonate buffer containing 100 ng of recombinant GST-(PDTRP)₇ fusion protein (Gendler *et al.*, 1990, *J. Mol. Biol.* 265:15286-93). After overnight binding at 4°C, the plate was washed three times in PBS-Tween (*i.e.* PBS containing 0.05% Tween-20). The plate was then blocked with
5 three 3-minute washes of PBS-Tween containing 1% BSA.

For each construct, 100 µl of supernatant was added to a well on the plate. In addition, hHMFG-1 of known concentration was serially diluted down the plate using doubling dilutions in 100 µl of PBS-Tween per well. The
10 plate was incubated for a further 1 h at 30°C, then 200 ng of MC135 anti-human kappa light chain antibody (binding site) in 100 µl of PBS-Tween was added to each well for 1 h at 30°C. After three 3-minute washes in PBS-Tween, 100 µl of anti-mouse IgG-peroxidase conjugate (Jackson 315-035-045), diluted 1:2000 in PBS-Tween, was added to each well and
15 incubated for 1 h at 30°C. Following a final set of three 3-minute washes in PBS-Tween, 100 µl of TMB substrate (Sigma) was added to each well of the plate and, after a colour developed, the optical density at 630 nm of the solution in each well of the plate was determined.

20 *Results*

(see Figure 22)

(D) Corrected bovine DNase I standard curves and DNase assay

25

DNase activity was determined using a modification of the methyl green-DNA complex degradation method (Sinicropi *et al.*, 1994, *Analyt. Biochem.* 222:351-358). Briefly, a 1:1 solution of the assay buffer and

methy green-salmon sperm DNA complex was mixed together to give a total volume of 0.2 ml. To this, 0.1 ml of tissue culture supernatant from transiently transfected CHO-L761h cells was added and the mixture incubated at 37°C. DNA cleavage by DNase results in a reduction in absorbance at 620 nm. Figure 23 shows a standard curve produced with various concentrations of bovine DNase I over a number a time point.

Figures 24 and 25 show DNase activity for the whole HMFG1 antibody- and F(ab')₂ - DNase fusions, respectively.

10

(E) Cytotoxicity of DNase constructs

Method

15 DNase constructs were transfected into CHO L761h cells using a calcium phosphate co-precipitation method (Gorman *et al.*, 1985, In: *DNA cloning* (2nd edition), Glover A(ed.), Academic Press, NY, 163-188). Included in the experiment were negative controls, consisting of cells transfected with TE buffer alone or with TE buffer and pEE6 expression vector. In addition to these controls, vectors that express hHMFG-1 (pAS6) and F(ab')₂ of hHMFG1 (both with specificity for PEM but without DNase I) were included.

25 The supernatant from these cells was harvested after 72 h of expression, followed by centrifugation to remove dead cells. MCF-7 cells were incubated for 1 h at 37°C with an aliquot of each of these supernatants. The amount of cellular lactate dehydrogenase (LDH) released from the MCF-7 cells due to the cytotoxicity of the supernatant was determined

using the CytoTox96 cytotoxic assay kit (Promega). Total lysis ('total LDH') was determined by measuring the target cell maximum LDH release using the kits lysis solution. The percentage of cells killed was then calculated as the proportion of the LDH released to the total LDH released. For each construct, the cytotoxicity assay was performed in quadruplicate, except for assay of pAS38 and 39, which were performed in triplicate. The values of LDH release for each construct were compared against either F(ab')₂ or whole antibody, or each other, using a one-tailed t-test in Excel.

10

Results

Figures 26 and 27 shows that there is negligible cell killing with either pAS6 (HMFG1 alone) or with pAS41 (F(ab')₂ alone). All of the hHMFG1 F(ab')₂-DNase I constructs kill significantly more cells than the F(ab')₂ fragment alone ($p < 0.00193$) and all of the antibody-DNase I constructs kill significantly more cells than antibody alone ($p < 0.00783$), except for perhaps pAS34 ($p < 0.021$).

20 (F) Use of the DNase-I/huHMFG-1 Fab fusion protein in the treatment of ovarian cancer

Patients diagnosed with ovarian cancer are treated by intravenous injection of the DNaseI/huHMFG-1 Fab fusion protein. Typically, a dose of between 1 to 100 mg will be administered weekly.

25

Therapeutic response is measured by the normal clinical procedures that are well known in the art, for example radio-imaging methods.

CLAIMS

1. A compound comprising a target cell-specific portion and a cytotoxic portion characterised in that:
 - (i) the target cell-specific portion comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof; and
 - (ii) the cytotoxic portion has endonucleolytic activity.
2. A compound according to Claim 1 wherein the target cell-specific portion comprises an humanised HMFG-1 antibody or an antigen binding fragment thereof.
3. A compound according to Claim 2 wherein the target cell-specific portion is an humanised HMFG-1 antibody.
4. A compound according to Claim 1 or 2 wherein the target cell-specific portion comprises an antigen binding fragment of the humanised antibody selected from the group consisting of Fab-like molecules, such as Fab and F(ab')₂, Fv molecules, disulphide-linked Fv molecules, ScFv molecules and single domain antibodies (dAbs).
5. A compound according to Claim 4 wherein the target cell-specific portion comprises a Fab molecule.
6. A compound according to Claim 4 wherein the target cell-specific

portion comprises a $F(ab')_2$ molecule.

7. A compound according to Claim 1 wherein the target cell-specific portion comprises at least part of one or both of the amino acid sequences of Figure 3.
8. A compound according to Claim 7 wherein the target cell-specific portion comprises both of the amino acid sequences of Figure 3.
9. A compound according to any one of Claims 1 to 8 wherein the cytotoxic portion has DNA endonucleolytic activity.
10. A compound according to Claim 9 wherein the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease.
11. A compound according to Claim 10 wherein the endonuclease is a mammalian deoxyribonuclease I.
12. A compound according to Claim 11 wherein the endonuclease is a human deoxyribonuclease I.
13. A compound according to Claim 1 wherein the endonuclease is a restriction endonuclease.
14. A compound according to Claim 10 wherein the cytotoxic portion comprises the amino acid sequence of Figure 2.
15. A compound according to any one of Claims 1 to 14 wherein a nuclear

localization signal is incorporated.

16. A compound according to Claim 15 wherein the nuclear localization signal comprises the sequence PKKKRKV.
17. A compound according to any one of Claims 1 to 16 wherein the target cell-specific portion and the cytotoxic portion are fused.
18. A compound according to Claim 17 wherein the target cell-specific portion and the cytotoxic portion are separated by a linker sequence.
19. A compound according to Claim 18 wherein the linker sequence is or comprises GG or GSGG.
20. A nucleic acid encoding a compound as defined in any one of Claims 17 to 19.
21. A vector comprising a nucleic acid according to Claim 20.
22. A host cell comprising a vector according to Claim 21.
23. A pharmaceutical composition comprising a compound according to any one of Claims 1 to 19 and a pharmaceutically acceptable carrier.
24. A compound according to any one of Claims 1 to 19 for use in medicine.
25. Use of a compound according to any one of Claims 1 to 19 in the

preparation of a medicament for treating a mammal having said target cells to be destroyed.

26. A method of treating a mammal having target cells to be destroyed, the method comprising administering a compound according to any one of Claims 1 to 19 to said mammal.
27. A use according to Claim 25 or a method according to Claim 26 wherein the mammal is a human.
27. A use according to Claim 25 or a method according to Claim 26 wherein the target cells to be destroyed are cancer cells.
28. A use or a method according to Claim 27 wherein the cancer cells are epithelial cancer cells.
29. A use or a method according to Claim 28 wherein the cancer cells are ovarian, gastric, colorectal and/or pancreatic cancer cells.
30. A use or a method according to Claim 29 wherein the cancer cells are ovarian cancer cells.
31. A compound substantially as described herein, preferably with reference to one or more of the accompanying figures.

40

ABSTRACT**Compounds for Targeting**

A compound comprising a target cell-specific portion and a cytotoxic portion characterised in that the target cell-specific portion comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof, and the cytotoxic portion has endonucleolytic activity. Preferably, the target cell-specific portion comprises an humanised HMFG-1 antibody or an antigen binding fragment thereof. Advantageously, the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease, *e.g.* a human DNA endonuclease I. The invention further provides nucleic acids encoding the compounds of the invention, and the use of such compounds in medicine, *e.g.* in the treatment of cancer.

~~FIGURE 2~~
no figure

ROBERT JOHNSON
2YES CONCEPT
01000 014000

519100

FIGURE 1Human DNase I

(complete mRNA)

LOCUS HUMDNASEI 1039 bp mRNA
 DEFINITION Human DNase I mRNA, complete cds. PRI 06-MAR-1995
 ACCESSION M55983
 VERSION M55983.1 GI:181623
 KEYWORDS DNase I.
 SOURCE Human pancreas, cDNA to mRNA.
 ORGANISM Homo sapiens
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
 Eutheria; Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 1039)
 AUTHORS Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis
 JOURNAL sputum
 MEDLINE Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192. (1990)
 FEATURES 91067672

source

Location/Qualifiers

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/organism="Homo sapiens"

/db_xref="taxon:9606"

/clone="hDNase-18-1"

/tissue_type="pancreas"

sig peptide

160..225

/gene="DNase I"

CDS

160..1008

/gene="DNase I"

/codon_start=1

/product="DNase I"

/protein_id="AAA63170.1"

/db_xref="GI:181624"

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 DAVAIEDALYDVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIRLWTSPTFQWLI
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 VEVLK"

gene

160..1008

/gene="DNase I"

mat peptide

226..1005

/gene="DNase I"

/product="DNase I"

BASE COUNT

226 a

305 c

282 g

226 t

ORIGIN

```

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61 ttttctttaa gcagcaaaag gagaaaattg tcatcaaaag atattccaga ttcttgacag
121 cattctcgtc atctctgagg acatcaccat catctcagga tgagggggcat gaagotgctg
181 ggggcgctgc tggcactggc ggccctactg caggggggocg tgctccctgaa gatcgagcc
241 ttcaacatcc agacatttgg ggagaccaag atgtccaatg ccaccctcgt cagctacatt
301 gtgcagatcc tgagccgcta tgacatcgcc ctgggtccagg aggtcagaga cagccacctg
361 actgccgtgg ggaagctgct ggacaacctc aatcaggatg caccagacac ctatcactac
421 gtggtcagtg agccactggg acggaacagc tataaggago gctacotgtt cgtgtacagg
481 cctgaccagg tgtctgagggt ggacagctac tactacgatg atggctgcga gcootgagg
541 aacgacacct tcaaccgaga gccagccatt gtcaggttct tctcccggtt cccagaggte
601 agggagtttg ccattgttcc cctgcatgcg gccccggggg acgcagtagc cgagatcgac
661 gctctctatg acgtctacct ggatgtccaa gagaaatggg gcttgaggga cgtcatgttg
721 atgggcgact tcaatgcggg ctgcagctat gtgagaccct cccagtggtc atccatccgc
781 ctgtggacaa gccccacott ccagtggtc atccccgaca ccgctgacac cacagctaca
841 cccacgcact gtgcctatga caggatcgtg gttgcaggga tgctgctccg aggcgcggtt
901 gttcccgact cggctcttcc ctttaacttc caggctgcct atggcctgag tgaccaactg

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1/84

961 gcccaagcca tcagtgaacca ctatccagtg gaggtgatgc tgaagtgage agcccctccc
1021 cacaccagtt gaactgcag

2/84

FIGURE 2

human DNase I construct

LOCUS hDNASE.DN 753 bp mRNA PRI 06-MAR-1995
 DEFINITION Human DNase I mRNA, complete cds, Mature sequence modified to remove Nari site
 ACCESSION M55983
 NID g181623
 KEYWORDS DNase I.
 SOURCE Human pancreas, cDNA to mRNA.
 ORGANISM Homo sapiens
 Eukaryota; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 1039)
 AUTHORS Shak, S., Capon, D.J., Hellmich, R., Marsters, S.A. and Baker, C.L.
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis
 sputum
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
 MEDLINE 91067672
 FEATURES
 source Location/Qualifiers
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 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 /clone="hDNase-18-1"
 /tissue_type="pancreas"
 sig_peptide 160..225
 CDS
 /gene="DNase I"
 160..1008
 /gene="DNase I"
 /codon_start=1
 /product="DNase I"
 /db_xref="PID:g181624"
 /translation="LKIAAFNIQITGETKMSNATLVSYIVQILSRVDIALVQEVDRSH
 LTAVGKLLDNLNQDAPDTYHYVSEPLGRNSYKERYLFVYKFDQVSAVDSEYYDDGCE
 PCGNDFNREPAIVREFTSRETEVREFAIIVPLHAAPGDVAVARIDALYDVLVDQEKWGL
 EDVNLMDFTNACCSYVRPSSQNSAIRLWTSPTFQNLIEDSADTTATPTHCAVDRIIVAG
 MLLRGAVVFDSELEFNFQAYGLSDQLAQATSDHYPVVEVMTK"
 gene 160..1008
 /gene="DNase I"
 mat_peptide 226..1005
 /gene="DNase I"
 /product="DNase I"
 BASE COUNT 168 a 236 c 220 g 159 t
 ORIGIN
 1 CTGAGATCG CAGCCITCAA CATCCAGACA TTTGGGGAGA CCAAGATGTC CAATGCCACC
 61 CTCGTACGCT ACATTGTGCA GATCCTGAGC CGCTACGACA TCGCCCTGGT CCAGGAGGTC
 121 ACAGACAGCC ACCTGACTGC CGTGGGGAAG CTGCTGGACA ACCTCAATCA GGACGCACCA
 181 CAGACCTATC ACTACGTCCT CAGTGAGCCA CTGGGACGGA ACAGCTATAA GGAGCGCTAC
 241 CTGTTCTGTG ACAGGCCTGA CCAGCTGTCT GCGGTGGACA GCTACTACTA CGATGATGGC
 301 TCCGAGCCCT GCGGGAACGA CACCTTCAAC CGAGAGCCAG CCATTGTGAG GTTCTTCTCC
 361 CGGTTACAC ACCTCAGGGA GTTTCGCAAT GTTCCCTGCG ATCCSCCCCG GGGGACCGCA
 421 CTACCCACGA TCGACGCTCT CTATGACGTC TACCTGGATG TCCAGAGAGAA ATGGGGCTTG
 481 GAGGACGTCA TGTGATGAG CGACTTCAAT GCGGGCTGCA CCTATGTCAG ACCCTGCCAG
 541 TCGTCATCCA TCGGCTGTG GACAAGCCCG ACCCTCCAGT GCGTGATCCC CGACAGCGCT
 601 GACACACAG CTACACCCAC GCACTGTGCC TATGACAGGA TCGTGGTTGC AGGGATGCTG
 661 CTCCGAGGCC CCTTCTTCC GCACTCGGCT CTTCCCTTTA ACTTCCAGGC TGCCATATGC
 721 CTGAGTGACC AACIGGCCCA AGCCATCAGT GACCACTATC CAGTGGAGGT CATCCTGAAC
 781 TGA

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Figure 3

(A) pAS6 - light chain

LOCUS HMFG1LC2.D 721 bp DNA
 DEFINITION HUMANISED HMFG1 LIGHT CHAIN VNP LEADER.
 ACCESSION
 KEYWORDS
 SOURCE
 ORGANISM
 REFERENCE 1 (BASES 1 TO 342)
 AUTHORS VERHOEYEN ET AL
 TITLE CONSTRUCTION OF RESHAPED HMFG1 ETC
 JOURNAL IMMUNOL. (1993):78, 364-370
 COMMENT SCANNED IN FROM JOURNAL
 FEATURES
 SITES

This is the sequence of the HMFG1 light chain gene with the
 vnp leader sequence attached. Translate from
 residue 1. Note residue 399 is T > A in all clones leading
 to R133 silent mutation (T in Verhoeven paper)

BASE COUNT 197 a 202 c 182 g 140 t
 ORIGIN (bp)

```

1 ATGGGATGGA GGTGTATCAT CCTCTCTCTG GTAGCAACAG CTACAGGTGT CCACTCCGAC
61 ATCCAGATGA CCCAGAGCCC AAGCAGCCIG AGCGCCAGCG TGGGTGACAG AGTCACCATC
121 ACCTGTAACT CCAGTCAGAG CCTTTTATAT ACTAGCAATC AAAAGATCTA CTGGGCTGG
181 TACGAGCAGA AGCCAGGTAA GGCTCCAAAG CTGCTGATCT ACTGGGCATC CACTAGGGAA
241 TCTGGTGTGC CAAGCAGATT CAGCGGTAGC GGTAGCCGCTA CCGACTTCAC CTTCACCATC
301 AGCAGCCTCC AGCCACAGGA CATCGCCACC TACTACTGCC AGCAATATTA TAGATATCTT
361 CCGACGTTCC GCCAAGGGAC CAAGGTCCAA ATCAAACCAA CTGTGGCTGC ACCATCTGTC
421 TTCATCTTCC CGCCATCTCA TGAGCAGTTC AAATCIGGAA CTGCTCTGT TGTGTGCTG
481 CTGAATAACT TCTATCCAG AGAGGCCAAN GTACAGTCCA AGGTGGATAA CGCCCTCCAA
541 TCCCTAACT CCGAGGAGAG TGTCACAGAG CAGGACAGCA AGGACAGCAC CTACAGCCTC
601 AGCAGCAGCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAGTCTA CGCCTCCGAA
661 GTCACCCATC AGGGCCTGAG CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTTAG
721 A
  
```

//

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FIGURE 3

(3) PAS6 - heavy chain

LOCUS RHMFG1HC.D 1404 bp DNA
 DEFINITION HUMANISED HMFG1 heavy chain
 ACCESSION RHMFG1H
 KEYWORDS
 SOURCE
 ORGANISM
 REFERENCE
 AUTHORS VERHOEYEN ET AL
 TITLE CONSTRUCTION OF RESHAPED HMFG1 etc
 JOURNAL IMMUNOL. (1993):78, 364-370
 COMMENT VH domain SCANNED IN FROM JOURNAL
 FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KADAT (I.E. V TO A)
 FEATURES Residue 963 is C > T leading to silent mutation in all clones
 SITES Note
 BASE COUNT 332 a 439 c 379 g 253 t
 ORIGIN

LEPPER.

```

1 ATGGGATGGA GCTGTATCAT CCTCTCTTGG GTAGCAACAG CTACAGGTTG CCACTCCAG
61 GTGCAGCTCG TGCACCTCTG GGCAGAGGTG AAAAGAGCTG GGCCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTGAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGCCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTICA AGGGCCGAGT GACAGTCACT ACAGACACAT CCACAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACIGTGCAAC ATCCTACGAC
361 TTTCCTGGT TTGCTTACTG GGGCCAGGG ACTCTGCTCA CAGTCTCCTC AGCCCTCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCTCTCAAGA GCACCTCTGG GGGCACAGCG
481 GGCCTGGGCT GCCTGGTCAA GGACTACTTC TCTCTCAAGA GCACCTCTGG GGGCACAGCG
541 GCGCGCCCTG CCACCCGCTT CACACCTTC CCGGAACCGG TGACGGTGTG GTGGAACTCA
601 TCCCTCAGCA GCGTGGTGAC CGTCCCTTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCGAG CACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAACTG ACACHTGCCC ACCGTGGCCA GCACCTGAA TCTTGGGGGG ACCCTCAGTC
781 TTCTCTTTC CCCCAGAAC CAAGGACAGG CTCATGATCT CCGGAGCCCT TGAGGTGACA
841 TGCGTGGTGG TGACAGTGAG CCACCAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GCGGTGGAGG TGATAATGC CAAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCAGGTAC
961 CCTGTGGTCA GCGTCTCAG CCGTCTGAC CAGGACTGGC TGAATGGCAA GGAGTACAG
1021 TGCAAGGTCT CCAACAAAGC GGTGTACACC CCGATCGAGA AACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAAGCACA GGTGTACACC CCGATCGAGA AACCATCTC CAAAGCCAAA
1141 AACAGGTCA GCCTGAGCTG CCGTCTGAC CCGATCGAGA AACCATCTC CAAAGCCAAA
1201 TGCAAGGTCT CCAACAAAGC GGTGTACACC CCGATCGAGA AACCATCTC CAAAGCCAAA
1261 CACGGCTCCT TCTTCTCTA CAGCAAGCTC ACCGTGGACA ACAGCAGGTG GCAGCAGGGG
1321 AACCTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCGGGTAA ATGA
  
```

Antibody DNA fusions made here (34→39.)

End of lower hinge region of heavy chain. PAPE amino
 acid seq. Fab₂ fusions were made at this point.

Those with HYBRID HINGES ARE ALTERED FURTHER
 UP

ie.

THIS PART GACAAACTGACACA
 IS → D K T H T

AFTER THIS SEQUENCE YOU GET THE
 HYBRID HINGE & LINKER SEQUENCES
 Then DNase I

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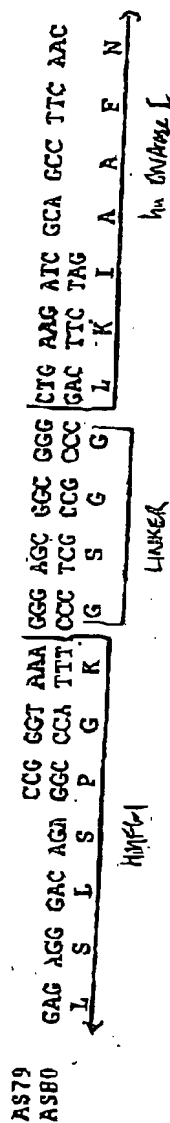
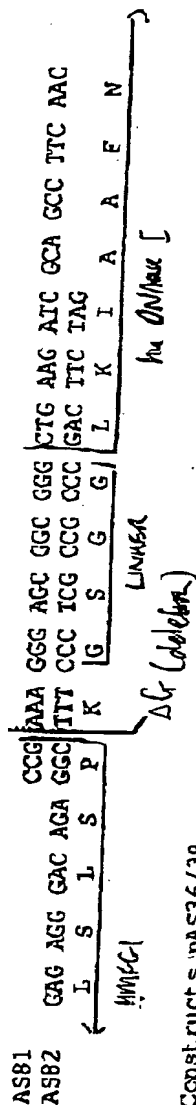
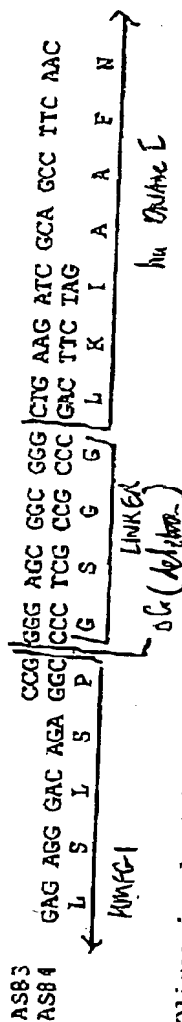
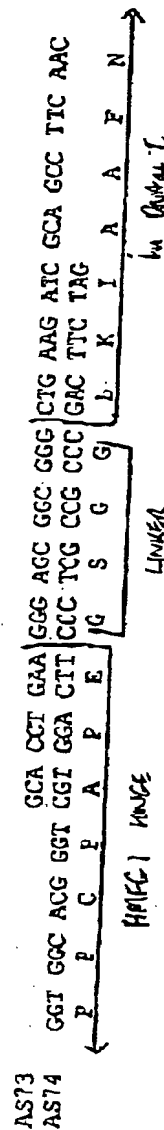
FIGURE 4Oligos Involved in the fusion of whole antibody-DNAseConstructs pAS34/37Constructs pAS35/38Constructs pAS36/39Oligos involved in the fusion of Fab'2-DNaseIConstructs pAS23/27

FIGURE 5

(A) pAS23

LOCUS PAS23.DNA 1554 bp mRNA PRI 06-MAR-1995
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (construct 1)
 ACCESSION
 NID
 KEYWORDS DNase I.
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f
 MHDNASE1.dna)
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo..
 AUTHORS Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis
 sputum
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
 MEDLINE 91067672
 BASE COUNT 344 a 468 c 434 g 308 t
 ORIGIN

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1 ATGGGATGCA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCACT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCACTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACCTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCTCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAACTC ACACATGCCC ACCGTGCCCC GCACCTGAAG GGAGCGCGCG GCTGAAGATC
781 GCAGCCTTCA ACATCCAGAC ATTTGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC
841 TACATTGTGC AGATCCTGAG CCGCTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC
901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT
961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCTGTTCTGTG
1021 TACAGGCCTG ACCAGGTGTC TGCGGTGGAC AGCTACTACT ACGATGATGG CTGCGAGCCC
1081 TGCGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTTCTTCTC CCGGTTTACA
1141 GAGGTCAGGG AGTTTGCCAT TGTTCCCTTG CATGCGGCCC CGGGGGACGC AGTAGCCGAG
1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGCTT GGAGGACGTC
1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC
1321 ATCCGCTGTG GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA
1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGTTG CAGGGATGCT GCTCCGAGGG
1441 GCCGTTGTTT CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC
1501 CAACTGGCCC AAGCCATCAG TGACCACTAT CCAGTGGAGG TGATGCTGAA GTGA

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File : PAS23.DNA

Range : 1 - 1554 Mode : Normal

Codon table : Universal

FIGURE 5(B)

```

      9      18      27      36      45      54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    ---
    M  G  W  S  C  I  I  L  F  L  V  A  T  A  T  G  V  H
      63      72      81      90      99      108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    ---
    S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S
      117      126      135      144      153      162
CTC AAC CTC TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    ---
    V  K  V  S  C  K  A  S  G  Y  T  F  S  A  Y  W  I  E
      171      180      189      198      207      216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    ---
    W  V  R  Q  A  P  G  K  G  L  E  W  V  G  E  I  L  P
      225      234      243      252      261      270
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    ---
    G  S  N  N  S  R  Y  N  E  K  F  K  G  R  V  T  V  T
      279      288      297      306      315      324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    ---
    R  D  T  S  T  N  T  A  Y  M  E  L  S  S  L  R  S  E
      333      342      351      360      369      378
CAC ACA CCC CTC TAT TAC TCT CCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    ---
    D  T  A  V  Y  Y  C  A  R  S  Y  D  F  A  W  F  A  Y
      387      396      405      414      423      432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    ---
    W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G  P  S
      441      450      459      468      477      486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    ---
    V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L
      495      504      513      522      531      540
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    ---
    G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S
      549      558      567      576      585      594
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    ---
    G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G

```

-1-

603	612	621	630	639	648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG					
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT					
V E P K S C D K T H T C P P C P A P					
765	774	783	792	801	810
GAA GGG AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG					
E G S G G L K I A A F N I Q T F G E					
819	828	837	846	855	864
ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC					
T K M S N A T L V S Y I V Q I L S R					
873	882	891	900	909	918
TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG					
Y D I A L V Q E V R D S H L T A V G					
927	936	945	954	963	972
AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC					
K L L D N L N Q D A P D T Y H Y V V					
981	990	999	1008	1017	1026
AGT GAC CCA CTC CCA CCG AAC ACC TAT AAG GAG CGC TAC CTG TTC GTC TAC ACC					
S E P L G R N S Y K E R Y L F V Y R					
1035	1044	1053	1062	1071	1080
CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC					
P D Q V S A V D S Y Y Y D D G C E P					
1089	1098	1107	1116	1125	1134
TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG					
C G N D T F N R E P A I V R F F S R					
1143	1152	1161	1170	1179	1188
TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC					
F T E V R E F A I V P L H A A P G D					
1197	1206	1215	1224	1233	1242

GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA

A V A E I D A L Y D V Y L D V Q E K

1251 1260 1269 1278 1287 1296
TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT

W G L E D V M L M G D F N A G C S Y

1305 1314 1323 1332 1341 1350
GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG

V R P S Q W S S I R L W T S P T F Q

1359 1368 1377 1386 1395 1404
TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT

W L I P D S A D T T A T P T H C A Y

1413 1422 1431 1440 1449 1458
GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG

D R I V V A G M L L R G A V V P D S

1467 1476 1485 1494 1503 1512
GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA

A L P F N F Q A A Y G L S D Q L A Q

1521 1530 1539 1548
GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'

A I S D H Y P V E V M L K *

FIGURE 6

(A) PAS27

LOCUS PAS27.DNA 1584 bp mRNA PRI 06-MAR-1995
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I with SV40 NLS (construct 1)
 ACCESSION
 NID
 KEYWORDS DNase I.
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f MHDNASE1.dna)
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 AUTHORS Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
 MEDLINE 91067672
 BASE COUNT 354 a 474 c 446 g 310 t
 ORIGIN

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAAGCTTG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGCTCTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCGGAACCGG TGACGGTGTC GTGGAAGTCA
541 GGCGCCCTGA CCAGCGCGGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC AUAAGUUUAG CAACACCAAG GTGGACAAGA AAGTTGACCC CAAATCTTGT
721 GACAAACTC ACACATGCCC ACCGTGCCCC GCACCTGAAG GGAGCGGCGG GCTGAAGATC
781 GCAGCCTTCA ACATCCAGAC ATTTGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC
841 TACATTGTCC AGATCCTGAG CCGCTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC
901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT
961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCTGTTCTGT
1021 TACAGGCCTG ACCAGGTGTC TCGGCTGGAC AGCTACTACT ACCATGATGG CTGCGAGCCC
1081 TCGGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTTCCTTCT CCGGTTTACA
1141 GAGGTCAGGG AGTTTGCCAT TGTTCCCTCT CATGCGGCCC CCGGGGACGC AGTAGCCGAG
1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGCTT GGAGQACGTC
1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC
1321 ATCCGCCTGT GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA
1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGGTTG CAGGGATGCT GCTCCGAGGG
1441 GCCGTTGTTC CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC
1501 CAACTGGCCC AAGCCATCAG TGACCACTAT CCAGTGGAGG TGATGCTGAA GGGGGCGCGA
1561 CCCAAAAAGA AGCGCAAGGT TTGA

```

L → NLS

File : PAS27.DNA

Range : 1 -

1584

Mode : Normal

Codon Table : Universal

FIGURE 6(B)

5'	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC	
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H	
	63					72			81			90			99			108	
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA	
	S	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	
	117					126			135			144			153			162	
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG	
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E	
	171					180			189			198			207			216	
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT	
	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	P	
	225					234			243			252			261			270	
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT	
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T	
	279					288			297			306			315			324	
	AGA	CAC	ACA	TCC	ACA	AAC	ACA	CCC	TAC	ATC	GAC	CTC	AGC	ACC	CTC	ACC	TCT	GAC	
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E	
	333					342			351			360			369			378	
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC	
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y	
	387					396			405			414			423			432	
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG	
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S	
	441					450			459			468			477			486	
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG	
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L	
	495					504			513			522			531			540	
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA	
	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	
	549					558			567			576			585			594	
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTG	CTA	CAC	TCC	TCA	CCA	
	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	

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603	612	621	630	639	648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG					
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT					
V E P K S C D K T H T C P P C P A P					
765	774	783	792	801	810
GAA GGG AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG					
E G S G G L K I A A F N I Q T F G E					
819	828	837	846	855	864
ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC					
T K M S N A T L V S Y I V Q I L S R					
873	882	891	900	909	918
TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG					
Y D I A L V Q E V R D S H L T A V C					
927	936	945	954	963	972
AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC					
K L L D N L N Q D A P D T Y H Y V V					
981	990	999	1008	1017	1026
AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG					
S E P L G R N S Y K E R Y L F V Y R					
1035	1044	1053	1062	1071	1080
CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC					
P D Q V S A V D S Y Y Y D D G C E P					
1089	1098	1107	1116	1125	1134
TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG					
C G N D T F N R E P A I V R F F S R					
1143	1152	1161	1170	1179	1188
TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT CCC CCC CCC CCC CAC					
F T E V R E F A I V F L H A A P G D					
1197	1206	1215	1224	1233	1242

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GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA

A V A E I D A L Y D V Y L D V Q E K

1251 1260 1269 1278 1287 1296
TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT

W G L E D V M L M G D F N A G C S Y

1305 1314 1323 1332 1341 1350
CTG AGA CCC TCC CAG TGG TCA TCC ATC CCC CTC TCG ACA AGC CCC ACC TTC CAG

V R P S Q W S S I R L W T S P T F Q

1359 1368 1377 1386 1395 1404
TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT

W L I P D S A D T T A T P T H C A Y

1413 1422 1431 1440 1449 1458
CAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CCA CCC CCC GTT GTT CCC GAC TCG

D R I V V A G M L L R G A V V P D S

1467 1476 1485 1494 1503 1512
GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA

A L P P N F O A A Y G L S D Q L A Q

1521 1530 1539 1548 1557 1566
GCC ATC AGT GAC CAC TAT CCA CTC CAC CTC ATC CTG AAG GGG GGC GGA CCC AAA

A I S D H Y P V E V M L K G G G P K

1575 1584
AAG AAG CGC AAG GTT TGA 3'

K K R K V *

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FIGURE 7**(A) pAS34**

LOCUS PAS34.DNA 2196 bp 2196 bp 2196 bp DNA 14-AUG-1998
 DEFINITION HUMANISED HMFG1 heavy chain fused to human DNase construct 34
 DEFINITION Clone 16.4.2 (same as hcdnasel.dna template file)
 REFERENCE
 AUTHORS VERHOEYEN ET AL
 TITLE CONSTRUCTION OF RESHAPED HMFG1 etc
 JOURNAL IMMUNOL. (1993):78, 364-370
 COMMENT Human DNase sequence is modified as a result of oligo assembly (mhdnase.dna)
 COMMENT The fusion was made using overlapping oligos AS79 and AS80
 FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
 FEATURES Residue 963 is G > T leading to silent mutation in all clones
 SITES Note
 BASE COUNT 501 a 677 c 607 g 411 t
 ORIGIN ?

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1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACCTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAATC ACACATGCCC ACCGTGCCCC GCACCTGAAC TCCTGGGGGG ACCGTGAGTC
781 TTCCTCTTCC CCCCCAAAAC CAAGGACACC CTATGATCT CCCGGACCCC TGAGGTCACA
841 TCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GCGGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA AGCAGCTAC
961 CGTGTGGTCA GCGTCCTCAC CGTCTGTCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGGGTAA AGGGAGCGGC GGGCTGAAGA TCGCAGCCTT CAACATCCAG
1441 ACATTGSGGG AGACCAAGAT GTCCAATGCC ACCCTCGTCA GCTACATTGT GCAGATCCTG
1501 AGCCGCTACG ACATCGCCCT GGTCCAGGAG GTCAGAGACA GCCACCTGAC TGCCGTGGGG
1561 AAGCTGCTGG ACAACCTCAA TCAGGACGCA CCAGACACCT ATCACTACGT GGTCAGTGAG
1621 CCACTGGGAC GGAACAGCTA TAAGGAGCGC TACCTGTTTG TGTACAGGCC TGACCAGGTG
1681 TCTGCGGTGG ACAGCTACTA CTACGATGAT GGCTGCGAGC CCTGCGGGAA CGACACCTTC
1741 AACCGAGAGC CAGCCATTGT CAGSTTCTTC TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC
1801 ATTGTTCCCC TGCATGCGGC CCCGGGGGAC GCAGTAGCCG AGATCGACGC TCTCTATGAC
1861 GTCTACCTGG ATGTCCAAGA GAAATGGGGC TTGGAGGACG TCATGTTGAT GGGCGACTTC
1921 AATGCGGGCT GCAGCTATGT GAGACCCTCC CAGTGGTCAT CCATCCGCTT GTGGACAAGC
1981 CCCACCTTCC AGTGGCTGAT CCCCAGACAG GCTGACACCA CAGCTACACC CACGCACTGT
2041 GCCTATGACA GGATCGTGGT TGCAGGGATG CTGCTCCGAG GGGCCGTTGT TCCCGACTCG
2101 GCTCTTCCCT TTAACCTCCA GGCTGCCTAT GGCCTGAGTG ACCAACTGGC CCAAGCCATC
2161 AGTGACCACT ATCCAGTGGA GGTGATGCTG AAGTGA

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File : PAS34.DNA
Range : 1 - 2196 Mode : Normal
Codon Table : Universal

FIGURE 7(B)

5'	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA
	S	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT
	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	D
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E
	CAC	ACA	CCC	GTC	TAT	TAC	TCT	CCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L
	CCC	TCC	CTC	CTC	AAC	GAC	TAC	TTC	CCC	CAA	CCC	CTC	ACC	CTG	TCC	TCC	AAC	TCA
	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA
	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G
	CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG
	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q
	657					666				675			684		693			702

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ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
T Y I C N V N H K P S N T K V D K K
711 720 729 738 747 756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
V E F K S C D K T H T C P P C P A P
765 774 783 792 801 810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
E L L G G P S V F L F P P K P K D T
819 828 837 846 855 864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GAC GTG AGC CAC
L M I S R T P E V T C V V V D V S H
873 882 891 900 909 918
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
E D P E V K P N W Y V D G V E V H N
927 936 945 954 963 972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
A K T K P R E E Q Y N S T Y R V V S
981 990 999 1008 1017 1026
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG
V L T V L H Q D W L N G K E Y K C K
1035 1044 1053 1062 1071 1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
V S N K A L P A P I E K T I S K A K
1089 1098 1107 1116 1125 1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
G Q P R E P Q V Y T L P P S R D E L
1143 1152 1161 1170 1179 1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
T K N Q V S L T C L V K G F Y P S D
1197 1206 1215 1224 1233 1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
I A V E W R S N G Q P E N N Y K T T
1251 1260 1269 1278 1287 1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
P P V L D S D G S F F L Y S K L T V
1305 1314 1323 1332 1341 1350
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
D K S R W Q Q G N V F S C S V M H E

1359	1368	1377	1386	1395	1404
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA GGG					
A L H N H Y T Q K S L S L S P G K G					
1413	1422	1431	1440	1449	1458
AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG					
S G G L K I A A F N I Q T F G E T K					
1467	1476	1485	1494	1503	1512
ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC					
M S N A T L V S Y I V Q I L S R Y D					
1521	1530	1539	1548	1557	1566
ATC CCC CTC CTC CAG CAG CTC AGA CAC AGC CAC CTG ACT GCC GTG GGG AAG CTC					
I A L V O E V R D S H L T A V G K L					
1575	1584	1593	1602	1611	1620
CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG					
L D N L N Q D A D D T Y H Y V V S E					
1629	1638	1647	1656	1665	1674
CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC					
P L G R N S Y K E R Y L F V Y R P D					
1683	1692	1701	1710	1719	1728
CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG					
Q V S A V D S Y Y Y D D G C E P C G					
1737	1746	1755	1764	1773	1782
AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA					
N D T F N R E P A I V R F F S R F T					
1791	1800	1809	1818	1827	1836
GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA					
E V R E F A I V P L H A A P G D A V					
1845	1854	1863	1872	1881	1890
GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC					
A E I D A L Y D V Y L D V Q E K W G					
1899	1908	1917	1926	1935	1944
TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA					
L E D V M L M G D F N A G C S Y V R					
1953	1962	1971	1980	1989	1998
CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG					
P S Q W S S I R L W T S P T F Q W L					
2007	2016	2025	2034	2043	2052
ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG					
I P D S A D T T A T P T H C A Y D R					

2061	2070	2079	2088	2097	2106
ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT					
I V V A G M L L R G A V V P D S A L					
2115	2124	2133	2142	2151	2160
CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG CCC CAA GCC ATC					
P F N F Q A A Y G L S D Q L A Q A I					
2169	2178	2187	2196		
AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'					
S D K Y P V E V M L K *					

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FIGURE 8

(A) pAS35

LOCUS PAS35.DNA 2193 bp 2193 bp DNA 14-AUG-1998
DEFINITION HUMANISED HMFG1 heavy chain fused to human DNase construct 35
DEFINITION Clone 17.12.1 with silent K to K mutation (1398 A > G)
REFERENCE
AUTHORS VERHOEYEN ET AL
TITLE CONSTRUCTION OF RESHAPED HMFG1 etc
JOURNAL IMMUNOL. (1993):78, 364-370
COMMENT Human DNase sequence is modified as a result of oligo assembly (mhdnase.dna)
COMMENT The fusion was made using overlapping oligos AS81 and AS82
FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
FEATURES Residue 963 is G > T leading to silent mutation in all clones
FEATURES In 17.12.1 residue 1398 is A > G (silent K to K mutation)
SITES Note
BASE COUNT 500 a 677 c 606 g 410 t
ORIGIN ?

```
1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GGCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAU UTACATCTGC
661 AACGTGAATC ACAGGCCAC CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAACTC ACACATGCCC ACCGTGCCCC GCACCTGAAC TCCTGGGGGG ACCCTCAGTC
781 TTCCTCTTCC CCCCCAAACC CAAGGACACC CTCATGATCT CCGGACCCCC TGAGGTCACA
841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
961 CGTGTGGTCA GCGTCCCTAC CGTCTGAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CTTCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCGGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCTCTTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGAAGGG GAGCGCCGGG CTGAAGATCG CAGCCTTCAA CATCCAGACA
1441 TTTGGGGAGA CCAAGATGTC CAATGCCACC CTCGTCAGCT ACATTGTGCA GATCCTGAGC
1501 CGCTACGACA TCGCCCTGGT CCAGGAGGTG AGAGACAGCC ACCTGACTGC CGTGGGGAAG
1561 CTGCTGGACA ACCTCAATCA GGACGCACCA GACACCTATC ACTACGTGGT CAGTGAGCCA
1621 CTGGGACGGA ACAGCTATAA GGAGCGCTAC CTGTTCTGT ACAGGCCTGA CCAGGTGTCT
1681 GCGGTGGACA GCTACTACTA CGATGATGGC TGCGAGCCCT GCGGGAACGA CACCTTCAAC
1741 CGAGAGCCAG CCATTGTCAG GTTCTTCTCC CGGTTACAG AGGTCAGGGA GTTTGCCATT
1801 GTTCCCTGTC ATGCGGCCCC GGGGGACGCA GTAGCCGAGA TCGACGCTCT CTATGACGTC
1861 TACCTGGATG TCCAAGAGAA ATGGGGCTTG GAGGACGTCA TGTTGATGG CGACTTCAAT
1921 GCGGGCTGCA GCTATGTGAG ACCCTCCCAG TGGTCATCCA TCCGCCTGTG GACAAGCCCC
1981 ACCTTCCAGT GGCTGATCCC CGACAGCGCT GACACCACAG CTACACCCAC GCACTGTGCC
2041 TATGACAGGA TCGTGGTTGC AGGGATGCTG CTCCGAGGGG CCGTTGTTCC CGACTCGGCT
2101 CTTCCCTTTA ACTTCCAGGC TGCCTATGGC CTGAGTGACC AACTGGCCCA AGCCATCAGT
2161 GACCACTATC CAGTGGAGGT GATGCTGAAG TGA
```

```
File : PAS35.DNA
Range : 1 - 2193    Mode : Normal
Codon Table : Universal
```

FIGURE 8(B)

5	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC	54
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H	
			63			72			81			90			99			108	
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA	
	S	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	
			117			126			135			144			153			162	
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG	
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E	
			171			180			189			198			207			216	
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT	
	W	V	R	Q	A	P	G	K	C	L	E	W	V	G	E	I	L	P	
			225			234			243			252			261			270	
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT	
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T	
			279			288			297			306			315			324	
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG	
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E	
			333			342			351			360			369			378	
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC	
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y	
			387			396			405			414			423			432	
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TGG	
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S	
			441			450			459			468			477			486	
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG	
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L	
			495			504			513			522			531			540	
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCC	GTC	ACC	GTC	TCC	TGG	AAC	TCA	
	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	
			549			558			567			576			585			594	
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA	
	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	
			603			612			621			630			639			648	
	CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG	
	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	C	T	Q	
			657			666			675			684			693			702	

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ACC TAC ATC TCC AAC CTC AAT CAC AAG CCC ACC AAC ACC AAC CTC CAC AAC AAA
T Y I C N V N H K P S N T K V D K K
711 720 729 738 747 756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
V E P K S C D K T H T C P P C P A P
765 774 783 792 801 810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
E L L G G P S V F L F P P K P K D T
819 828 837 846 855 864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG CTC GAC GTC AGC CAC
L M I S R T P E V T C V V V D V S H
873 882 891 900 909 918
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
E D P E V K F N W Y V D G V E V H N
927 936 945 954 963 972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
A K T K P R E E Q Y N S T Y R V V S
981 990 999 1008 1017 1026
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG
V L T V L H Q D W L N G K E Y K C K
1035 1044 1053 1062 1071 1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
V S N K A L P A P I E K T I S K A K
1089 1098 1107 1116 1125 1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
G Q P R E P Q V Y T L P P S R D E L
1143 1152 1161 1170 1179 1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
T K N Q V S L T C L V K G F Y P S D
1197 1206 1215 1224 1233 1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
I A V E W E S N G Q P E N N Y K T T
1251 1260 1269 1278 1287 1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
P P V L D S D G S P P L Y S K L T V
1305 1314 1323 1332 1341 1350
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
D K S R W Q Q G N V F S C S V M H E

1359	1368	1377	1386	1395	1404
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG AAG GGG AGC					
A L H N H Y T Q K S L S L S P K G S					
1413	1422	1431	1440	1449	1458
GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG					
G G L K I A A F N I Q T F C E T K M					
1467	1476	1485	1494	1503	1512
TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC ATC					
S N A T L V S Y I V Q I L S R Y D I					
1521	1530	1539	1548	1557	1566
GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG CTG					
A L V Q E V R D S H L T A V G K L L					
1575	1584	1593	1602	1611	1620
GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA					
D N L N Q D A P D T Y H Y V V S E P					
1629	1638	1647	1656	1665	1674
CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC CAG					
L G R N S Y K E R Y L F V Y R P D Q					
1683	1692	1701	1710	1719	1728
GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG AAC					
V S A V D S Y Y Y D D G C E F C G N					
1737	1746	1755	1764	1773	1782
GAC ACC TTC AAC CCA GAG CCA GCC ATT GTC AGC TTC TTC TCC CGG TTC ACA GAG					
D T F N R E P A I V R F F S R F T E					
1791	1800	1809	1818	1827	1836
GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC					
V R E F A I V P L H A A P G D A V A					
1845	1854	1863	1872	1881	1890
GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC TTG					
E I D A L Y D V Y L D V Q E K W G L					
1899	1908	1917	1926	1935	1944
GAG GAC GTC ATC TTG ATC CCC CAC TTC AAT CCC GCC TGC ACC TAT CTC ACA CCC					
E D V M L M G D F N A G C S Y V R P					
1953	1962	1971	1980	1989	1998
TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG ATC					
S Q W S S I R L W T S P T F Q W L I					
2007	2016	2025	2034	2043	2052
CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC					
P D S A D T T A T P T H C A Y D R I					

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2061	2070	2079	2088	2097	2106
GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC					
V V A C M L L R G A V V P D S A L F					
2115	2124	2133	2142	2151	2160
TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT					
F N F Q A A Y G L S D Q L A Q A I S					
2169	2178	2187			
GAC CAC TAT CCA CTC GAC CTC ATC CTC AAC TCA 3'					
D H Y P V E V M L K *					

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FIGURE 9

(A) pAS36

LOCUS PAS36.DNA 2190 bp 2190 bp DNA 14-AUG-1998
 DEFINITION HUMANISED HMFG1 heavy chain fused to human DNase - construct 36
 DEFINITION Clone 18.24.1 with residue 1392 T > C
 REFERENCE
 AUTHORS VERHOEYEN ET AL
 TITLE CONSTRUCTION OF RESHAPED HMFG1 etc
 JOURNAL IMMUNOL. (1993):78, 364-370
 COMMENT Human DNase sequence is modified as a result of oligo assembly (mndnase.dna)
 COMMENT The fusion was made using overlapping oligos AS83 and AS84
 FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
 FEATURES Residue 963 is G > T leading to silent mutation in all clones
 FEATURES Residue 1392 T > C silent S to S mutation
 SITES Note
 BASE COUNT 498 a 678 c 605 g 409 t
 ORIGIN ?

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAGGGGCC TCGAGTGGGT CCGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCGGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGGTGTC GTGGAACCTA
541 GCGCGCCTGA CCAGCGCGCT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAATC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GCGTGGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
961 CGTGTGGTCA GCGTCTTCAC CGTCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGAGACC GGAGAACAAC TACAAGACCA CGCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CCGCGGGGAG CCGCGGGCTG AAGATCGCAG CCTTCAACAT CCAGACATTT
1441 GGGGAGACCA AGATGTCCAA TGCCACCCTC GTCAGCTACA TTGTGCAGAT CCTGAGCCGC
1501 TACGACATCG CCCTGGTCCA GGAGGTCAGA GACAGCCACC TGAATGCCGT GGGGAAGCTG
1561 CTGGACAACC TCAATCAGGA CGCACCAGAC ACCTATCACT ACGTGGTCAG TGAGCCACTG
1621 GGACGGAACA GCTATAAGGA GCGCTACCTG TTCGTGTACA GGCTGACCA GGTGTCTGCG
1681 GTGGACAGCT ACTACTACGA TGATGGCTGC GAGCCCTGCG GGAACGACAC CTTCAACCGA
1741 GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG TTCACAGAGG TCAGGGAGTT TGCCATTGTT
1801 CCCCCTGCATG CGGCCCGGGG GGACGCAGTA GCCGAGATCG ACGCTCTCTA TGACGTCTAC
1861 CTGGATGTCC AAGAGAAATG GGGCTTGGAG GACGTCATGT TGATGGGCGA CTTCAATGCG
1921 GGCTGCAGCT ATGTGAGACC CTCCAGTGG TCATCCATCC GCCTGTGGAC AAGCCCCACC
1981 TTCCAGTGGC TGATCCCCGA CAGCGCTGAC ACCACAGCTA CACCCACGCA CTGTGCCTAT
2041 GACAGGATCG TGGTTGCAGG GATGCTGCTC CGAGGGGCGG TTGTTCCCGA CTCGGCTCTT
2101 CCCTTTAACT TCCAGGCTGC CTATGGCCTG AGTGACCAAC TGGCCCAAGC CATCAGTGAC
2161 CACTATCCAG TGGAGGTGAT GCTGAAGTGA

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File : PAS36.DNA

Range : 1 - 2190 Mode : Normal

Codon Table : Universal

FIGURE 9 (B)

```

      9      18      27      36      45      54
5' ATG CCA TCC ACC TCT ATC ATC CTC TTC TTC CTA GCA ACA GCT ACA GGT GTC CAC
   ---
   M G W S C I I L F L V A T A T G V H

      63      72      81      90      99      108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
   ---
   S Q V Q L V Q S G A E V K K P G A S

      117      126      135      144      153      162
GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
   ---
   V K V S C K A S G Y T F S A Y W I E

      171      180      189      198      207      216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
   ---
   W V R Q A P G K G L E W V G E I L P

      225      234      243      252      261      270
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
   ---
   G S N N S R Y N E K F K G R V T V T

      279      288      297      306      315      324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
   ---
   R D T S T N T A Y M E L S S L R S E

      333      342      351      360      369      378
GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
   ---
   D T A V Y Y C A R S Y D F A W F A Y

      387      396      405      414      423      432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
   ---
   W G Q G T L V T V S S A S T K G P S

      441      450      459      468      477      486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
   ---
   V F P L A P S S K S T S G G T A A L

      495      504      513      522      531      540
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
   ---
   G C L V K D Y F P E P V T V S W N S

      549      558      567      576      585      594
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
   ---
   G A L T S G V H T F P A V L Q S S G

      603      612      621      630      639      648
CTC TAC TCC CTC AGC AGC GTG GTG ACC CTC CCC TCC ACC AGC TTG GGC ACC CAG
   ---
   L Y S L S S V V T V P S S S L G T O

      657      666      675      684      693      702

```

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ACC	TAC	ATC	TGC	AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG	GTG	GAC	AAG	AAA				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
T	Y	I	C	N	V	N	H	K	P	S	N	T	K	V	D	K	K				
711				720				729				738				747				756	
GTT	GAG	CCC	AAA	TCT	TGT	GAC	AAA	ACT	CAC	ACA	TGC	CCA	CCG	TGC	CCA	GCA	CCT				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
V	E	P	K	S	C	D	K	T	H	T	C	P	P	C	P	A	P				
765				774				783				792				801				810	
GAA	CTC	CTG	GGG	GGA	CCG	TCA	GTC	TTC	CTC	TTC	CCC	CCA	AAA	CCC	AAG	GAC	ACC				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
E	L	L	G	G	P	S	V	F	L	F	P	P	K	P	K	D	T				
819				828				837				846				855				864	
CTC	ATG	ATC	TCC	CGG	ACC	CCT	GAG	CTC	ACA	TCC	CTG	CTC	CTG	GAC	CTG	ACC	CAC				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
L	M	I	S	R	T	P	E	V	T	C	V	V	V	D	V	S	H				
873				882				891				900				909				918	
GAA	GAC	CCT	GAG	GTC	AAG	TTC	AAC	TGG	TAC	GTG	GAC	GGC	GTG	GAG	GTG	CAT	AAT				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
E	D	P	E	V	K	F	N	W	Y	V	D	C	V	E	V	H	N				
927				936				945				954				963				972	
GCC	AAG	ACA	AAG	CCG	CGG	GAG	GAG	CAG	TAC	AAC	AGC	ACG	TAC	CGT	GTG	GTC	AGC				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
A	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S				
981				990				999				1008				1017				1026	
GTC	CTC	ACC	GTC	CTG	CAC	CAG	GAC	TGG	CTG	AAT	GGC	AAG	GAG	TAC	AAG	TGC	AAG				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K				
1035				1044				1053				1062				1071				1080	
GTC	TCC	AAC	AAA	GCC	CTC	CCA	GCC	CCC	ATC	GAG	AAA	ACC	ATC	TCC	AAA	GCC	AAA				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K				
1089				1098				1107				1116				1125				1134	
GGG	CAG	CCC	CGA	GAA	CCA	CAG	GTG	TAC	ACC	CTG	CCC	CCA	TCC	CGG	GAT	GAG	CTG				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L				
1143				1152				1161				1170				1179				1188	
ACC	AAG	AAC	CAG	GTC	AGC	CTG	ACC	TGC	CTG	GTC	AAA	GGC	TTC	TAT	CCC	AGC	GAC				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
T	K	N	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D				
1197				1206				1215				1224				1233				1242	
ATC	GCC	GTG	GAG	TGG	CAG	ACC	AAT	CCC	CAG	CCC	CAC	AAC	AAC	TAC	AAG	ACC	ACG				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
I	A	V	E	W	E	S	N	G	O	P	E	N	N	Y	K	T	T				
1251				1260				1269				1278				1287				1296	
CCT	CCC	GTG	CTG	GAC	TCC	GAC	GGC	TCC	TTC	TTC	CTC	TAC	AGC	AAG	CTC	ACC	GTG				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
P	P	V	L	D	S	D	G	S	F	F	L	Y	S	K	L	T	V				
1305				1314				1323				1332				1341				1350	
GAC	AAG	AGC	AGG	TGG	CAG	CAG	GGG	AAC	GTC	TTC	TCA	TGC	TCC	GTG	ATG	CAT	GAG				
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E				

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1359				1368				1377				1386				1395				1404			
GCT	CTG	CAC	AAC	CAC	TAC	ACG	CAG	AAG	AGC	CTC	TCC	CTG	TCC	CCG	GGG	AGC	GGC						
A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	S	G						
1413				1422				1431				1440				1449				1458			
GGG	CTG	AAG	ATC	GCA	GCC	TTC	AAC	ATC	CAG	ACA	TTT	GGG	GAG	ACC	AAG	ATG	TCC						
G	L	K	I	A	A	F	N	I	Q	T	F	G	E	T	K	M	S						
1467				1476				1485				1494				1503				1512			
AAT	GCC	ACC	CTC	GTC	AGC	TAC	ATT	GTG	CAG	ATC	CTG	AGC	CGC	TAC	GAC	ATC	GCC						
N	A	T	L	V	S	Y	I	V	Q	I	L	S	R	Y	D	I	A						
1521				1530				1539				1548				1557				1566			
CTG	GTC	CAG	GAG	GTC	AGA	GAC	AGC	CAC	CTG	ACT	GCC	GTG	GGG	AAG	CTG	CTG	GAC						
L	V	Q	E	V	R	D	S	H	L	T	A	V	G	K	L	L	D						
1575				1584				1593				1602				1611				1620			
AAC	CTC	AAT	CAG	GAC	GCA	CCA	GAC	ACC	TAT	CAC	TAC	GTG	GTC	AGT	GAG	CCA	CTG						
N	L	N	Q	D	A	P	D	T	Y	H	Y	V	V	S	E	P	L						
1629				1638				1647				1656				1665				1674			
GGA	CGG	AAC	AGC	TAT	AAG	GAG	CGC	TAC	CTG	TTC	GTG	TAC	AGG	CCT	GAC	CAG	GTG						
G	R	N	S	Y	K	E	R	Y	L	F	V	Y	R	P	D	Q	V						
1683				1692				1701				1710				1719				1728			
TCT	GCG	GTG	GAC	AGC	TAC	TAC	TAC	GAT	GAT	GGC	TGC	GAG	CCC	TGC	GGG	AAC	GAC						
S	A	V	D	S	Y	Y	Y	D	D	G	C	E	P	C	G	N	D						
1737				1746				1755				1764				1773				1782			
ACC	TTC	AAC	CGA	GAG	CCA	GCC	ATT	GTC	AGG	TTC	TTC	TCC	CGG	TTC	ACA	GAG	GTC						
T	F	N	R	E	P	A	I	V	R	F	F	S	R	F	T	E	V						
1791				1800				1809				1818				1827				1836			
AGG	GAG	TTT	GCC	ATT	GTT	CCC	CTG	CAT	GCG	GCC	CCG	GGG	GAC	GCA	GTA	GCC	GAG						
R	E	F	A	I	V	P	L	H	A	A	P	G	D	A	V	A	R						
1845				1854				1863				1872				1881				1890			
ATC	GAC	GCT	CTC	TAT	GAC	GTC	TAC	CTG	GAT	GTC	CAA	GAG	AAA	TGG	GGC	TTG	GAG						
I	D	A	L	Y	D	V	Y	L	D	V	Q	E	K	W	G	L	E						
1899				1908				1917				1926				1935				1944			
GAC	GTC	ATG	TTG	ATG	GGC	GAC	TTC	AAT	GCG	GGC	TGC	AGC	TAT	GTG	AGA	CCC	TCC						
D	V	M	L	M	G	D	F	N	A	G	C	S	Y	V	R	P	S						
1953				1962				1971				1980				1989				1998			
CAG	TGG	TCA	TCC	ATC	CGC	CTG	TGG	ACA	AGC	CCC	ACC	TTC	CAG	TGG	CTG	ATC	CCC						
Q	W	S	S	I	R	L	W	T	S	P	T	F	Q	W	L	I	P						
2007				2016				2025				2034				2043				2052			
GAC	AGC	GCT	GAC	ACC	ACA	GCT	ACA	CCC	ACG	CAC	TGT	GCC	TAT	GAC	AGG	ATC	GTG						
D	S	A	D	T	T	A	T	P	T	H	C	A	Y	D	R	I	V						

2061	2070	2079	2088	2097	2106
GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC TTT					
V A G M L L R G A V V P D S A L P F					
2115	2124	2133	2142	2151	2160
AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT GAC					
N F Q A A Y G L S D Q L A Q A I S D					
2169	2178	2187			
CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'					
H Y P V E V M L K *					

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30
/ 24

FIGURE 10

(A) pAS37

LOCUS PAS37.DNA 2226 bp 2196 bp DNA 14-AUG-1998
 DEFINITION HUMANISED HMFG1 heavy chain fused to human DNase construct 37
 DEFINITION Clone 16.4.2 (same as hcdnasel.dna template file) plus NLS
 REFERENCE
 AUTHORS VERHOEYEN ET AL
 TITLE CONSTRUCTION OF RESHAPED HMFG1 etc
 JOURNAL IMMUNOL. (1993):78, 364-370
 COMMENT Human DNase sequence is modified as a result of oligo assembly (mhdnase.dna)
 COMMENT The fusion was made using overlapping oligos AS79 and AS80
 FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
 FEATURES Residue 963 is G > T leading to silent mutation in all clones
 SITES Note
 BASE COUNT 511 a 683 c 619 g 413 t
 ORIGIN ?

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAAGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGAATACTTC CCGCAACCGG TGACGGTGTC GTGGAAC TCA
541 GCGGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAACTC ACACATGCCC ACCGTGCCCC GCACCTGAAC TCCTGGGGGG ACCGTCAGT
781 TTCCTCTTCC CCCCCAAACC CAAGGACACC CTCATGATCT CCCGACCCC TGAGGTCACA
841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GCGGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
961 CGTGTGGTCA GCGTCTCTAC CGTCTGACAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT GCCTGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGGGTAA AGGGAGCGGC GGGCTGAAGA TCGCAGCCTT CAACATCCAG
1441 ACATTTGGGG AGACCAAGAT GTCCAATGCC ACCCTCGTCA GCTACATTGT GCAGATCCTG
1501 AGCCGCTACG ACATCGCCCT GGTCCAGGAG GTCAGAGACA GCCACCTGAC TGCCGTGGGG
1561 AAGCTGCTGG ACAACCTCAA TCAGGACGCA CCAGACACCT ATCACTACGT GGTCACTGAG
1621 CCACTGGGAC GGAACAGCTA TAAGGAGCGC TACCTGTTCG TGTACAGGCC TGACCAGGTG
1681 TCTGCGGTGG ACAGCTACTA CTACGATGAT GGCTGCGAGC CCTGCGGGAA CGACACCTTC
1741 AACCAGAGGC CAGCCATTGT CAGGTTCTTC TCCCGGTTCA CAGAGGTGAG GGAGTTTGCC
1801 ATTGTTCCCC TGCATGCGGC CCGGGGGGAC GCAGTAGCCG AGATCGACGC TCTCTATGAC
1861 GTCTACCTGG ATGTCCAAGA GAAATGGGGC TTGGAGGACG TCATGTTGAT GGGCGACTTC
1921 AATGCGGGCT GCAGCTATGT GAGACCCTCC CAGTGGTCAAT CCATCCGCCT GTGGACAAGC
1981 CCCACCTTCC AGTGGCTGAT CCCCAGACAGC GCTGACACCA CAGCTACACC CACGCACTGT
2041 GCCTATGACA GGATCGTGGT TGCAGGGATG CTGCTCCGAG GGGCCGTTGT TCCCGACTCG
2101 GCTCTTCCCT TTAACCTCCA GGCTGCCTAT GGCCTGAGTG ACCCACTGGC CCAAGCCATC
2161 AGTGACCACT ATCCAGTGGA GGTGATGCTG AAGGGGGGCG GACCCAAAAA GAAGCGCAAG
2221 GTTTGA

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L NLS

File : PAS37.DNA
Range : 1 - 2226 Mode : Normal
Codon Table : Universal

FIGURE 10 (B)

```

      9      18      27      36      45      54
5' ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
   ---
   M G W S C I I L F L V A T A T G V H

      63      72      81      90      99      108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG CTG AAA AAG CCT CCG CCC TCA
   ---
   S O V Q L V Q S G A E V K K P G A S

      117      126      135      144      153      162
GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
   ---
   V K V S C K A S G Y T F S A Y W I R

      171      180      189      198      207      216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
   ---
   W V R Q A P G K G L E W V G E I L P

      225      234      243      252      261      270
GGA AGT AAT AAT TCT ACA TAC AAT CAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
   ---
   G S N N S R Y N E K F K G R V T V T

      279      288      297      306      315      324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
   ---
   R D T S T N T A Y M E L S S L R S E

      333      342      351      360      369      378
GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
   ---
   D T A V Y Y C A R S Y D F A W F A Y

      387      396      405      414      423      432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
   ---
   W G Q G T L V T V S S A S T K G P S

      441      450      459      468      477      486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
   ---
   V F P L A P S S K S T S G G T A A L

      495      504      513      522      531      540
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
   ---
   G C L V K D Y F P E P V T V S W N S

      549      558      567      576      585      594
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
   ---
   G A L T S G V H T F P A V L Q S S G

      603      612      621      630      639      648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
   ---
   L Y S L S S V V T V P S S S L G T Q

      657      666      675      684      693      702

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32
84

ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
T Y I C N V N H K P S N T K V D K K

711 720 729 738 747 756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
V E P K S C D K T H T C P P C P A P

765 774 783 792 801 810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
E L L G G P S V F L F P P K P K D T

819 828 837 846 855 864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC
L M I S R T P E V T C V V V D V S H

873 882 891 900 909 918
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
E D P E V K F N W Y V D G V E V H N

927 936 945 954 963 972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
A K T K P R E E Q Y N S T Y R V V S

981 990 999 1008 1017 1026
CTC CTC ACC CTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG
V L T V L H Q D W L N G K E Y K C K

1035 1044 1053 1062 1071 1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
V S N K A L P A P I E K T I S K A K

1089 1098 1107 1116 1125 1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
G Q P R E P Q V Y T L F P S R D E L

1143 1152 1161 1170 1179 1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
T K N Q V S L T C L V K G F Y P S D

1197 1206 1215 1224 1233 1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
I A V E W E S N G Q P E N N Y K T T

1251 1260 1269 1278 1287 1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
P P V L D S D C S F F L Y S K L T V

1305 1314 1323 1332 1341 1350
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
D K S R W Q Q G N V F S C S V M H E

1359	1368	1377	1386	1395	1404
GCT CTG CAC AAC CAC TAC ACC CAC AAC ACC CTC TCC CTC TCT CCC GGT AAA CCC					
A L H N H Y T O K S L S L S P G K G					
1413	1422	1431	1440	1449	1458
AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG					
S O G L K I A A F N I Q T F G E T K					
1467	1476	1485	1494	1503	1512
ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC					
M S N A T L V S Y I V Q I L S R Y D					
1521	1530	1539	1548	1557	1566
ATC CCC CTG CTC CAC GAC GTC AGA GAC AGC CAC CTG ACT GCC GTG GGC AAG CTC					
I A L V Q E V R D S H L T A V G K L					
1575	1584	1593	1602	1611	1620
CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG					
L D N L N Q D A D D T Y H Y V V S E					
1629	1638	1647	1656	1665	1674
CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC					
P L G R N S Y K E R Y L F V Y R P D					
1683	1692	1701	1710	1719	1728
CAC GTC TCT GCG GTG GAC AGC TAC TAC TAC CAT CAT CCC TGC GAG CCC TGC GGC					
Q V S A V D S Y Y Y D D G C E P C G					
1737	1746	1755	1764	1773	1782
AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA					
N D T F N R E P A I V R F F S R F T					
1791	1800	1809	1818	1827	1836
GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA					
E V R E F A I V P L H A A P G D A V					
1845	1854	1863	1872	1881	1890
GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC					
A E I D A L Y D V Y L D V Q E K W G					
1899	1908	1917	1926	1935	1944
TTC GAG CAC CTC ATC TTG ATC GCG GAC TTC AAT GCG GCG TCC ACC TAT GTG AGA					
L E D V M L M G D F N A G C S Y V R					
1953	1962	1971	1980	1989	1998
CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG					
P S Q W S S I R L W T S P T F Q W L					
2007	2016	2025	2034	2043	2052
ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG					
I P D S A D T T A T P T H C A Y D R					

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34
82

2061	2070	2079	2088	2097	2106
ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT					
I V V A G M L L R G A V V P D S A L					
2115	2124	2133	2142	2151	2160
CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC					
P F N F Q A A Y G L S D Q L A Q A I					
2169	2178	2187	2196	2205	2214
AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG					
S D H Y P V E V M L K G G G P K K K					
2223					
CGC AAG GTT TGA 3'					
R K V *					

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FIGURE 11

(A) pAS38

LOCUS PAS38.DNA 2223 bp 2193 bp DNA 14-AUG-1998
DEFINITION HUMANISED HMFG1 heavy chain fused to human DNase construct 38
DEFINITION Clone 17.12.1 with silent K to K mutation (1398 A > G)+NLS
REFERENCE
AUTHORS VERHOEYEN ET AL
TITLE CONSTRUCTION OF RESHAPED HMFG1 etc
JOURNAL IMMUNOL. (1993):78, 364-370
COMMENT Human DNase sequence is modified as a result of oligo assembly (mhdnase.dna)
COMMENT The fusion was made using overlapping oligos AS81 and AS82
FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
FEATURES Residue 963 is G > T leading to silent mutation in all clones
FEATURES In 17.12.1 residue 1398 is A > G (silent K to K mutation)
SITES Note
BASE COUNT 510 a 683 c 618 g 412 t
ORIGIN ?

```
1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCACTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCITGG TTGCTTACTG GGGCCAAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAAGTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCTC AGGACTCTAC
601 TCCCTCAGCA CGGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAACTC ACACATGCCC ACCGTGCCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCAAC
841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
961 CGTGTGGTCA GCGTCCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCCAGAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCTCTTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGAAgGG GAGCGGCGGG CTGAAGATCG CAGCCTTCAA CATCCAGACA
1441 TTTGGGGAGA CCAAGATGTC CAATGCCACC CTCGTCAGCT ACATTGTGCA GATCCTGAGC
1501 CGCTACGACA TCGCCCTGGT CCAGGAGGTC AGAGACAGCC ACCTGACTGC CGTGGGGGAG
1561 CTGCTGGACA ACCTCAATCA GGACGCACCA GACACCTATC ACTACGTGGT CAGTGAGCCA
1621 CTGGGACGGA ACAGCTATAA GGAGCGCTAC CTGTTCTGTG ACAGGCCTGA CCAGGTGTCT
1681 GCGGTGGACA GCTACTACTA CGATGATGGC TGCGAGCCCT GCGGGAACGA CACCTTCAAC
1741 CGAGAGCCAG CCATTGTGAG GTTCTTCTCC CGGTTCACAG AGGTGAGGGA GTTTGCCATT
1801 GTTCCCCTGC ATGCGGCCCC GGGGGACGCA GTAGCCGAGA TCGACGCTCT CTATGACGTC
1861 TACCTGGATG TCCAAGAGAA ATGGGGCTTG GAGGACGTCA TGTTGATGGG CGACTTCAAT
1921 GCGGGCTGCA GCTATGTGAG ACCCTCCAGT TGGTCAATCA TCCGCCTGTG GACAAGCCCC
1981 ACCTTCCAGT GGCTGATCCC CGACAGCGCT GACACCCACG CTACACCCAC GCAGTGTGCC
2041 TATGACAGGA TCGTGGTTGC AGGGATGCTG CTCCGAGGGG CCGTTGTTCC CGACTCGGCT
2101 CTTCCCTTTA ACTTCCAGGC TGCTATGGC CTGAGTGACC AACTGGCCCA AGCCATCAGT
2161 GACCACTATC CAGTGGAGGT GATGCTGAAG GGGGGCGGAC CCAAAAAGAA GCGCAAGGTT
2221 TGA
```

L3MLS

File : PAS38.DNA
Range : 1 - 2223 Mode : Normal
Codon Table : Universal

FIGURE 11 (B)

			9			18			27			36			45			54
5'	ATG	OGA	TGG	ACC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H
			63			72			81			90			99			108
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAC	CTC	AAA	AAG	CCT	CGG	CCC	TCA
	S	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S
			117			126			135			144			153			162
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E
			171			180			189			198			207			216
	TCC	GTG	CGC	CAC	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT
	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	P
			225			234			243			252			261			270
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAC	CCC	CGA	GTG	ACA	GTC	ACT
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T
			279			288			297			306			315			324
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E
			333			342			351			360			369			378
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y
			387			396			405			414			423			432
	TGG	GGC	CAA	GGG	ACT	CTC	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S
			441			450			459			468			477			486
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	CCC	CCC	CTG
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L
			495			504			513			522			531			540
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA
	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S
			549			558			567			576			585			594
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA
	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G
			603			612			621			630			639			648
	CTC	TAC	TCC	CTC	AGC	ACC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG
	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q
			657			666			675			684			693			702

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ACC	TAC	ATC	TGC	AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG	GTG	CAC	AAG	AAA				
T	Y	I	C	N	V	N	H	K	P	S	N	T	K	V	D	K	K				

711				720				729				738				747				756	
GTT	GAG	CCC	AAA	TCT	TGT	GAC	AAA	ACT	CAC	ACA	TGC	CCA	CCG	TGC	CCA	GCA	CCT				
V	E	P	K	S	C	D	K	T	H	T	C	P	P	C	P	A	P				

765				774				783				792				801				810	
GAA	CTC	CTG	GGG	GGA	CCG	TCA	GTC	TTC	CTC	TTC	CCC	CCA	AAA	CCC	AAG	GAC	ACC				
E	L	L	G	G	P	S	V	F	L	F	P	P	K	P	K	D	T				

819				828				837				846				855				864	
CTC	ATG	ATC	TCC	CGG	ACC	CCT	GAG	GTC	ACA	TGC	GTG	GTG	GTG	GAC	GTG	AGC	CAC				
L	M	I	S	R	T	P	E	V	T	C	V	V	V	D	V	S	H				

873				882				891				900				909				918	
GAA	CAC	CCT	GAG	GTC	AAG	TTC	AAC	TGG	TAC	GTG	GAC	GGC	GTG	GAG	GTG	CAT	AAT				
E	D	P	E	V	K	F	N	W	Y	V	D	G	V	E	V	H	N				

927				936				945				954				963				972	
GCC	AAG	ACA	AAG	CCG	CGG	GAG	GAG	CAG	TAC	AAC	AGC	ACG	TAC	CGT	GTG	GTC	AGC				
A	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V	V	S				

981				990				999				1008				1017				1026	
GTC	CTC	ACC	GTC	CTG	CAC	CAG	GAC	TGG	CTG	AAT	GGC	AAG	GAG	TAC	AAG	TGC	AAG				
V	L	T	V	L	H	Q	D	W	L	N	C	K	E	Y	K	C	K				

1035				1044				1053				1062				1071				1080	
GTC	TCC	AAC	AAA	GCC	CTC	CCA	GCC	CCC	ATC	GAG	AAA	ACC	ATC	TCC	AAA	GCC	AAA				
V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K				

1089				1098				1107				1116				1125				1134	
GGG	CAG	CCC	CGA	GAA	CCA	CAG	GTG	TAC	ACC	CTG	CCC	CCA	TCC	CGG	GAT	GAG	CTG				
G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L				

1143				1152				1161				1170				1179				1188	
ACC	AAG	AAC	CAG	GTC	AGC	CTG	ACC	TGC	CTG	GTC	AAA	GGC	TTC	TAT	CCC	AGC	GAC				
T	K	N	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D				

1197				1206				1215				1224				1233				1242	
ATC	GCC	GTG	GAG	TGG	GAG	AGC	AAT	GGG	CAG	CCG	GAG	AAC	AAC	TAC	AAG	ACC	ACG				
I	A	V	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T				

1251				1260				1269				1278				1287				1296	
CCT	CCC	GTG	CTG	GAC	TCC	GAC	GGC	TCC	TTC	TTC	CTC	TAC	AGC	AAG	CTC	ACC	GTG				
P	P	V	L	D	S	D	G	S	F	F	L	Y	S	K	L	T	V				

1305				1314				1323				1332				1341				1350	
CAC	AAG	ACC	ACC	TCC	CAC	CAC	GGG	AAC	GTC	TTC	TCA	TCC	TCC	GTG	ATG	CAT	GAG				
D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M	H	E				

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1359	1368	1377	1386	1395	1404
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG AAG GGG AGC					
A L H N H Y T Q K S L S L S P K G S					
1413	1422	1431	1440	1449	1458
GGC GGG CTG AAC ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG					
G G L K I A A F N I Q T F G E T K M					
1467	1476	1485	1494	1503	1512
TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG ACC CGC TAC GAC ATC					
S N A T L V S Y I V Q I L S R Y D I					
1521	1530	1539	1548	1557	1566
GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG CTG					
A L V Q E V R D S H L T A V C K L L					
1575	1584	1593	1602	1611	1620
GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA					
D N L N Q D A P D T Y H Y V V S E F					
1629	1638	1647	1656	1665	1674
CTG GGA CGG AAC AGC TAT AAC CAG CCC TAC CTG TTC GTG TAC AGG CCT GAC CAG					
L G R N S Y K E R Y L F V Y R P D Q					
1683	1692	1701	1710	1719	1728
GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG AAC					
V S A V D S Y Y Y D D C C E P C C N					
1737	1746	1755	1764	1773	1782
GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA GAG					
D T F N R E P A I V R F F S R F T E					
1791	1800	1809	1818	1827	1836
GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC					
V R E F A I V P L H A A P G D A V A					
1845	1854	1863	1872	1881	1890
GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC TTG					
E I D A L Y D V Y L D V Q E K W G L					
1899	1908	1917	1926	1935	1944
GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA CCC					
E D V M L M G D F N A C C S Y V R P					
1953	1962	1971	1980	1989	1998
TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG ATC					
S Q W S S I R L W T S P T F Q W L I					
2007	2016	2025	2034	2043	2052
CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC					
P D S A D T T A T P T H C A Y D R I					

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2061	2070	2079	2088	2097	2106
GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC					
V V A G M L L R G A V V P D S A L P					
2115	2124	2133	2142	2151	2160
TMT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT					
F N F Q A A Y G L S D Q L A Q A I S					
2169	2178	2187	2196	2205	2214
GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG CGC					
D H Y P V E V M L K G G G P K K K R					
2223					
AAG GTT TGA 3'					
K V					

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FIGURE 12

(4) pAS39

LOCUS PAS39.DNA 2220 bp 2190 bp DNA 14-AUG-1998
 DEFINITION HUMANISED HMFG1 heavy chain fused to human DNase - construct 39
 DEFINITION Clone 18.24.1 with residue 1392 T > C +NLS
 REFERENCE
 AUTHORS VERHOEYEN ET AL
 TITLE CONSTRUCTION OF RESHAPED HMFG1 etc
 JOURNAL IMMUNOL. (1993):78, 364-370
 COMMENT Human DNase sequence is modified as a result of oligo assembly
 (mhdnase.dna)
 COMMENT The fusion was made using overlapping oligos AS83 and AS84
 FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
 FEATURES Residue 963 is G > T leading to silent mutation in all clones
 FEATURES Residue 1392 T > C silent S to S mutation
 SITES Note
 BASE COUNT 508 a 684 c 617 g 411 t
 ORIGIN ?

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1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTGCTCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAActCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAACTC ACACATGCCC ACCGTGCCCC GCACCTGAAC TCCTGGGGGG ACCGTGAGTC
781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CCGCGGGAG CGGCGGGCTG AAGATCGCAG CCTTCAACAT CCAGACATTT
1441 GGGGAGACCA AGATGTCCAA TGCCACCCTC GTCAGCTACA TTGTGCAGAT CCTGAGCCGC
1501 TACGACATCG CCCTGGTCCA GGAGGTGAGA GACAGCCACC TGAAGTCCGT GGGGAAGCTG
1561 CTGGACAACC TCAATCAGGA CGCACCAGAC ACCTATCACT ACGTGGTCAG TGAGCCACTG
1621 GGACGGAAAC GCTATAAGGA GCGTACCTG TTCGTGTACA GGCCTGACCA GGTGTCTGCG
1681 GTGGACAGCT ACTACTACGA TGATGGCTGC GAGCCCTGCG GGAACGACAC CTTCAACCGA
1741 GAGCCAGCCA TTGTGAGGTT CTTCTCCCGG TTCACAGAGG TCAGGGAGTT TGCCATTGTT
1801 CCCCTGCATG CGGCCCGGGG GGACGCAGTA GCCGAGATCG ACGCTCTCTA TGACGTCTAC
1861 CTGGATGTCC AAGAGAAATG GGGCTTGGAG GACGTCATGT TGATGGGCGA CTTCAATGCG
1921 GGCTGCAGCT ATGTGAGACC CTCCCAGTGG TCATCCATCC GCCTGTGGAC AAGCCCCACC
1981 TTCTAGTGGC TGATCCCCGA CAGCGCTGAG ACCACAGCTA CACCCACGCA CTGTGCCATAT
2041 GACAGGATCG TGGTTGCAGG GATGCTGCTC CGAGGGGCGG TTGTTCCCGA CTCGGCTCTT
2101 CCCTTTAACT TCCAGGCTGC CTATGGCCTG AGTGACCAAC TGGCCCAAGC CATCAGTGAC
2161 CACTATCCAG TGGAGGTGAT GCTGAAGGGG GGCGGACCCA AAAAGAAGCG CAAGGTTTGA

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File : PAS39.DNA
 Range : 1 - 2220 Mode : Normal
 Codon Table : Universal

FIGURE 12(B)

	9				18				27				36				45				54			
5'	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC						
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H						
	63				72				81				90				99				108			
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGC	CCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA						
	S	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S						
	117				126				135				144				153				162			
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG						
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E						
	171				180				189				198				207				216			
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT						
	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	P						
	225				234				243				252				261				270			
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	CAC	AAG	TTC	AAG	GGC	CCA	GTG	ACA	GTC	ACT						
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T						
	279				288				297				306				315				324			
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG						
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E						
	333				342				351				360				369				378			
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC						
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y						
	387				396				405				414				423				432			
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG						
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S						
	441				450				459				468				477				486			
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG						
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L						
	495				504				513				522				531				540			
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA						
	G	C	L	V	K	D	Y	F	D	E	D	V	T	V	S	W	N	S						
	549				558				567				576				585				594			
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA						
	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G						
	603				612				621				630				639				648			
	CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG						
	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q						
	657				666				675				684				693				702			

```
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG CAC AAC AAA
-----
T Y I C N V N H K P S N T K V D K K

711 720 729 738 747 756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
-----
V E P K S C D K T H T C P P C P A P

765 774 783 792 801 810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
-----
E L L G G P S V F L F P P K P K D T

819 828 837 846 855 864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC CTC ACC CAC
-----
L M I S R T P E V T C V V V D V S H

873 882 891 900 909 918
GAA CAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
-----
E D P E V K F N W Y V D G V E V H N

927 936 945 954 963 972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
-----
A K T K P R E E Q Y N S T Y R V V S

981 990 999 1008 1017 1026
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAC TCC AAG
-----
V L T V L H Q D W L N C K E Y K C K

1035 1044 1053 1062 1071 1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
-----
V S N K A L P A P I E K T I S K A K

1089 1098 1107 1116 1125 1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
-----
G Q P R E P Q V Y T L P P S R D E L

1143 1152 1161 1170 1179 1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
-----
T K N Q V S L T C L V K G F Y P S D

1197 1206 1215 1224 1233 1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
-----
I A V E W E S N G Q P E N N Y K T T

1251 1260 1269 1278 1287 1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
-----
P P V L D S D G G F F L Y S K L T V

1305 1314 1323 1332 1341 1350
GAC AAG AGC AGC TCC CAC CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
-----
D K S R W Q Q G N V F S C S V M H E
```

B
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1359	1368	1377	1386	1395	1404
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCC CCC GGG AGC GCC					
A L H N H Y T Q K S L S L S P G S G					
1413	1422	1431	1440	1449	1458
GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG TCC					
G L K I A A F N I Q T F G E T K M S					
1467	1476	1485	1494	1503	1512
AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC ATC GCC					
N A T L V S Y I V Q I L S R Y D I A					
1521	1530	1539	1548	1557	1566
CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG CGC AAC CTG CTG GAC					
L V Q E V R D S H L T A V G K L L D					
1575	1584	1593	1602	1611	1620
AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA CTG					
N L N Q D A P D T Y H Y V V S E P L					
1629	1638	1647	1656	1665	1674
GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC CAG GTG					
G R N S Y K E R Y L F V Y R P D Q V					
1683	1692	1701	1710	1719	1728
TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT CCC TGC GAG CCC TCC GCG AAC GAC					
S A V D S Y Y Y D D G C E P C G N D					
1737	1746	1755	1764	1773	1782
ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA GAG GTC					
T F N R E P A I V R F F S R F T E V					
1791	1800	1809	1818	1827	1836
AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC GAG					
R E F A I V P L H A A P G D A V A E					
1845	1854	1863	1872	1881	1890
ATC CAC CCT CTC TAT GAC GTC TAC CTC GAT GTC CAA GAG AAA TGG GGC TTG GAG					
I D A L Y D V Y L D V Q E K W G L E					
1899	1908	1917	1926	1935	1944
GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA CCC TCC					
D V M L M G D F N A G C S Y V R P S					
1953	1962	1971	1980	1989	1998
CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG ATC CCC					
Q W S S I R L W T S P T F Q W L J P					
2007	2016	2025	2034	2043	2052
GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC GTG					
D S A D T T A T P T H C A Y D R I V					

2061	2070	2079	2088	2097	2106
GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC TTT					
V A C M L L R G A V V P D S A L P F					
2115	2124	2133	2142	2151	2160
AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT GAC					
N F Q A A Y G L S D Q L A Q A I S D					
2169	2178	2187	2196	2205	2214
CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG CGC AAG					
H Y P V E V M L K G G G P K K K R K					

GTT TGA 3'

V *

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FIGURE 13

(A) PAS101

LOCUS PAS101.DNA 1548 bp mRNA PRI 06-MAR-1995
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (PAS101)
 ACCESSION
 NID
 KEYWORDS DNase I.
 SOURCE DNase I sequence is from assembled oligos (thus modified c/i
 MHDNASE1.dna)
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 AUTHORS Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis
 sputum
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
 MEDLINE 91067672
 BASE COUNT 343 a 467 c 430 g 308 t
 ORIGIN

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1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCACTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAACATC ACACATGCCC ACCGTGCCCC GCACCTGAAAG GCGGGCTGAA GATCGCAGCC
781 TTCAACATCC AGACATTGCG GGAGACCAAG ATGTCCAATG CCACCCTCGT CAGCTACATT
841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTCAGAGA CAGCCACCTG
901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG CACCAGACAC CTATCACTAC
961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG
1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG
1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC
1141 AGGGAGTTTG CCATTGTTCC CCTGCATGCG GCCCCGGGGG ACGCAGTAGC CGAGATCGAC
1201 GCTCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG
1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCTT CCCAGTGGTC ATCCATCCGC
1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA
1381 CCCACGCACT GTGCCATGA CAGGATCGTG GTTGCAGGGA TGCTGCTCCG AGGGGCCGTT
1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG
1501 GCCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGTGA

```

File : PAS101.DNA
 Range : 1 - 1548 Mode : Normal
 Codon Table : Universal

FIGURE 13(B)

```

      9      18      27      36      45      54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    -----
    M  G  W  S  C  I  I  L  F  L  V  A  T  A  T  C  V  H

      63      72      81      90      99      108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    -----
    S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S

      117      126      135      144      153      162
GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    -----
    V  K  V  S  C  K  A  S  G  Y  T  F  S  A  Y  W  I  E

      171      180      189      198      207      216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    -----
    W  V  R  O  A  P  G  K  G  L  E  W  V  G  E  I  L  P

      225      234      243      252      261      270
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    -----
    G  S  N  N  S  R  Y  N  E  K  F  K  G  R  V  T  V  T

      279      288      297      306      315      324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG ACC TCT CAG
    -----
    R  D  T  S  T  N  T  A  Y  M  E  L  S  S  L  R  S  E

      333      342      351      360      369      378
GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    -----
    D  T  A  V  Y  Y  C  A  R  S  Y  D  F  A  W  F  A  Y

      387      396      405      414      423      432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    -----
    W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G  P  S

      441      450      459      468      477      486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    -----
    V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L

      495      504      513      522      531      540
CCC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    -----
    G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S

      549      558      567      576      585      594
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAC TCC TCA GGA
    -----
    G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G
  
```

- 1 -

42
87

603	612	621	630	639	648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG					
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
CTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT					
V E P K S C D K T H T C P P C P A P					
765	774	783	792	801	810
GAA GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG					
E G G L K I A A F N I Q T F G E T K					
819	828	837	846	855	864
ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC					
M S N A T L V S Y I V Q I L S R Y D					
873	882	891	900	909	918
ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG					
I A L V Q E V R D S H L T A V G K L					
927	936	945	954	963	972
CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG					
L D N L N Q D A P D T Y H Y V V S E					
981	990	999	1008	1017	1026
CCA CTC CCA CCC AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC					
P L C R N S Y K E R Y L F V Y R P D					
1035	1044	1053	1062	1071	1080
CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG					
Q V S A V D S Y Y Y D D G C E F C G					
1089	1098	1107	1116	1125	1134
AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA					
N D T F N R E P A I V R F F S R F T					
1143	1152	1161	1170	1179	1188
GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA					
E V R E F A I V P L H A A P G D A V					
1197	1206	1215	1224	1233	1242

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CCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG CCC

A E I D A L Y D V Y L D V Q E K W G

1251 1260 1269 1278 1287 1296
TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA

L E D V M L M G D F N A G C S Y V R

1305 1314 1323 1332 1341 1350
CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG

P S Q W S S I R L W T S P T F Q W L

1359 1369 1377 1386 1395 1404
ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG

I P D S A D T T A T P T H C A Y D R

1413 1422 1431 1440 1449 1458
ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCC CCT CTT

I V V A G M L L R G A V V P D S A L

1467 1476 1485 1494 1503 1512
CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC

P F N F Q A A Y G L S D Q L A Q A I

1521 1530 1539 1548
AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'

S D H Y P V E V M L K *

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FIGURE 14

(A) pAS102

LOCUS PAS102.DNA 1566 bp mRNA PRI 06-MAR-1995
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS102)
 ACCESSION
 NID
 KEYWORDS DNase I.
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f
 MHDNASE1.dna) (see Figure 2)
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 AUTHORS Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis
 sputum
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
 MEDLINE 91067672
 BASE COUNT 345 a 469 c 440 g 312 t
 ORIGIN

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1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCACTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTIONTC CCCGAACCGG TGACGGTGTC GTGGAAGTCA
541 GGCGCCCTGA CCAAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCTCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAATC ACACATGCTG TGTGCACTGC CCACCGTGCC CAGCACCTCA AGGCACCGCC
781 GGGCTGAAGA TCGCAGCCTT CAACATCCAG ACATTTGGGG AGACCAAGAT GTCCAATGCC
841 ACCCTCGTCA GCTACATTGT GCAGATCCTG AGCCGCTACG ACATCGCCCT GGTCCAGGAG
901 GTCAGAGACA GCCACCTGAC TGCCGTGGGG AAGCTGCTGG ACAACCTCAA TCAGGACGCA
961 CCAGACACCT ATCACTACGT GGTCAGTGAQ CCACTGGGAC GGAACAGCTA TAAGGAGCGC
1021 TACCTGTTTG TGTACAGGCC TGACCAGGTG TCTGCGGTGG ACAGCTACTA CTACGATGAT
1081 GGCTGCGAGC CCTGCGGGAA CGACACCTTC AACCGAGAGC CAGCCATTGT CAGGTTCTTC
1141 TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC ATTGTTCCCC TGCATGCGGC CCCGGGGGAC
1201 GCAGTAGCCG AGATCGACGC TCTCTATGAC GTCTACCTGG ATGTCCAAGA GAAATGGGGC
1261 TTGGAGGACG TCATGTTGAT GGGCGACTTC AATGCGGGCT GCAGCTATGT GAGACCTCC
1321 CAGTGGTCAT CCATCCGCCT GTGGACAAGC CCCACCTTCC AGTGGCTGAT CCCCAGACAGC
1381 GCTGACACCA CAGCTACACC CACGCACTGT GCCTATGACA GGATCGTGGT TGCAGGGATG
1441 CTGCTCCGAG GGGCCGTTGT TCCCGACTCG GCTCTCCCT TTAATTCCA GGCTGCCTAT
1501 GGCCTGAGTG ACCAACTGGC CCAAGCCATC AGTGACCACT ATCCAGTGGA GGTGATGCTG
1561 AAGTGA

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50
84

File : PAS102.DNA
 Range : 1 - 1566 Mode : Normal
 Codon Table : Universal

FIGURE 14 (B)

```

      9      18      27      36      45      54
5' ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
   ---
   M  G  W  S  C  I  I  L  F  L  V  A  T  A  T  G  V  H

      63      72      81      90      99      108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
   ---
   S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S

      117      126      135      144      153      162
GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA CAG
   ---
   V  K  V  S  C  K  A  S  G  Y  T  F  S  A  Y  W  I  E

      171      180      189      198      207      216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
   ---
   W  V  R  Q  A  P  G  K  G  L  E  W  V  G  E  I  L  P

      225      234      243      252      261      270
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
   ---
   G  S  N  N  S  R  Y  N  E  K  F  K  G  R  V  T  V  T

      279      288      297      306      315      324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
   ---
   R  D  T  S  T  N  T  A  Y  M  E  L  S  S  L  R  S  E

      333      342      351      360      369      378
GAC ACA GCC GTC TAT TAC TGT GCA ACA TCC TAC GAC TTT GCC TGC TTT CCT TAC
   ---
   D  T  A  V  Y  Y  C  A  R  S  Y  D  F  A  W  F  A  Y

      387      396      405      414      423      432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
   ---
   W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G  P  S

      441      450      459      468      477      486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
   ---
   V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L

      495      504      513      522      531      540
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
   ---
   G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S

      549      558      567      576      585      594
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
   ---
   G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G

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- 1 -

SI
84

603				612				621				630				639				648			
CTC	TAC	TCC	CTC	AGC	ACC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG						
L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q						
657				666				675				684				693				702			
ACC	TAC	ATC	TGC	AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAC	CTG	GAC	AAC	AAA						
T	Y	I	C	N	V	N	H	K	P	S	N	T	K	V	D	K	K						
711				720				729				738				747				756			
GTT	GAG	CCC	AAA	TCT	TCT	GAC	AAA	ACT	CAC	ACA	TGC	TGT	GTG	GAG	TGC	CCA	CCG						
V	E	P	K	S	C	D	K	T	H	T	C	C	V	E	C	P	P						
765				774				783				792				801				810			
TGC	CCA	GCA	CCT	GAA	GGG	AGC	GGC	GGG	CTG	AAG	ATC	GCA	GCC	TTC	AAC	ATC	CAG						
C	P	A	P	E	G	S	G	G	L	K	I	A	A	F	N	I	Q						
819				828				837				846				855				864			
ACA	TTT	GGG	GAG	ACC	AAG	ATG	TCC	AAT	GCC	ACC	CTC	GTC	AGC	TAC	ATT	GTG	CAG						
T	F	G	E	T	K	M	S	N	A	T	L	V	S	Y	I	V	Q						
873				882				891				900				909				918			
ATC	CTG	AGC	CGC	TAC	GAC	ATC	GCC	CTG	CTC	CAG	GAG	GTC	ACA	CAC	AGC	CAC	CTG						
I	L	S	R	Y	D	I	A	L	V	Q	E	V	R	D	S	H	L						
927				936				945				954				963				972			
ACT	GCC	GTG	GGG	AAG	CTG	CTG	GAC	AAC	CTC	AAT	CAG	GAC	GCA	CCA	GAC	ACC	TAT						
T	A	V	G	K	L	L	D	N	L	N	Q	D	A	P	D	T	Y						
981				990				999				1008				1017				1026			
CAC	TAC	GTG	GTC	AGT	GAG	CCA	CTG	GGA	CGG	AAC	AGC	TAT	AAG	GAG	CGC	TAC	CTG						
H	Y	V	V	S	E	P	L	G	R	N	S	Y	K	E	R	Y	L						
1035				1044				1053				1062				1071				1080			
TTC	GTG	TAC	AGG	CCT	GAC	CAG	GTG	TCT	GCG	GTG	GAC	AGC	TAC	TAC	TAC	GAT	GAT						
F	V	Y	R	P	D	Q	V	S	A	V	D	S	Y	Y	Y	D	D						
1089				1098				1107				1116				1125				1134			
GGC	TGC	GAG	CCC	TGC	GGG	AAC	GAC	ACC	TTC	AAC	CGA	GAG	CCA	GCC	ATT	GTC	AGG						
G	C	E	P	C	C	N	D	T	F	N	R	E	P	A	I	V	R						
1143				1152				1161				1170				1179				1188			
TTC	TTC	TCC	CGG	TTC	ACA	CAC	CTC	AGG	GAG	TTT	CCC	ATT	GTT	CCC	CTG	CAT	GCG						
F	F	S	R	F	T	E	V	R	E	F	A	I	V	P	L	H	A						
1197				1206				1215				1224				1233				1242			

CCC CCG GGC GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT

A P G D A V A E I D A L Y D V Y L D

1251 1260 1269 1278 1287 1296
GTC CAA GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG

V Q E K W G L E D V M L M G D F N A

1305 1314 1323 1332 1341 1350
GGC TGC AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC

G C S Y V R P S Q W S S I R L W T S

1359 1368 1377 1386 1395 1404
CCC ACC TTC CAG TGG CTC ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG

P T F Q W L I P D S A D T T A T P T

1413 1422 1431 1440 1449 1458
CAC TGT GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTC CTC CGA GCG CCC GTT

H C A Y D R I V V A G M L L R G A V

1467 1476 1485 1494 1503 1512
GTT CCC GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC

V P D S A L P F N F Q A A Y G L S D

1521 1530 1539 1548 1557 1566
CAA CTG GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'

Q L A Q A I S D H Y P V E V M L K *

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FIGURE 15

(M) pAS103

LOCUS PAS103.DNA 1560 bp mRNA PRI 06-MAR-1995
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS103)
 ACCESSION
 NID
 KEYWORDS DNase I.
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f
 MHDNASE1.dna)
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 AUTHORS Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis
 sputum
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
 MEDLINE 91067672
 BASE COUNT 344 a 468 c 436 g 312 t
 ORIGIN

```

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAACA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAAGTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTCCTCAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCGAG CAACACCAAG GTGGACAAGA AACTTCAGCC CAAATCTTGT
721 GACAAAACCTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGCGGGCTG
781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC
841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA
901 GACAGCCACC TGACTGCCGT GGGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC
961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG
1021 TTCGTGTACA GGCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC
1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG
1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA
1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG
1261 GACGTCTATG TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG
1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC
1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC
1441 CGAGGGGGCG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG
1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGTGA

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54
84

File : PAS103.DNA
 Range : 1 - 1560 Mode : Normal
 Codon Table : Universal

FIGURE 15 (B)

```

      9      18      27      36      45      54
5' ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
   ---
   M  G  W  S  C  I  I  L  F  L  V  A  T  A  T  G  V  H

      63      72      81      90      99      108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
   ---
   S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S

      117      126      135      144      153      162
GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
   ---
   V  K  V  S  C  K  A  S  G  Y  T  F  S  A  Y  W  I  E

      171      180      189      198      207      216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
   ---
   W  V  R  Q  A  P  G  K  G  L  E  W  V  G  E  I  L  P

      225      234      243      252      261      270
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
   ---
   G  S  N  N  S  R  Y  N  E  K  F  K  G  R  V  T  V  T

      279      288      297      306      315      324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
   ---
   R  D  T  S  T  N  T  A  Y  M  E  L  S  S  L  R  S  E

      333      342      351      360      369      378
GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT CCC TGG TTT GCT TAC
   ---
   D  T  A  V  Y  Y  C  A  R  S  Y  D  F  A  W  F  A  Y

      387      396      405      414      423      432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
   ---
   W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G  F  S

      441      450      459      468      477      486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
   ---
   V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L

      495      504      513      522      531      540
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
   ---
   G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S

      549      558      567      576      585      594
GGC GCC CTG ACC ACC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
   ---
   G  A  L  T  S  C  V  H  T  F  P  A  V  L  Q  S  S  C

```

- 1 -

55/84

603	612	621	630	639	648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG					
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT CAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCG					
V E P K S C D K T H T C C V E C P P					
765	774	783	792	801	810
TGC CCA GCA CCT GAA GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT					
C P A P E G G L K I A A F N I Q T F					
819	828	837	846	855	864
GGG GAG ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG					
G E T K M S N A T L V S Y I V Q I L					
873	882	891	900	909	918
AGC CGC TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC					
S R Y D I A L V Q E V R D S H L T A					
927	936	945	954	963	972
GTG GGG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC					
V G K L L D N L N Q D A P D T Y H Y					
981	990	999	1008	1017	1026
GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG					
V V S E P L G R N S Y K E R Y L F V					
1035	1044	1053	1062	1071	1080
TAC AGG CCT GAC CAG GTG TCT CCC CTC GAC AGC TAC TAC TAC CAT CAT CCG TGC					
Y R P D Q V S A V D S Y Y Y D D G C					
1089	1098	1107	1116	1125	1134
GAG CCC TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC					
E P C G N D T F N R E P A I V R F F					
1143	1152	1161	1170	1179	1188
TCC CGG TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG					
S R P T E V R E F A I V P L H A A P					
1197	1206	1215	1224	1233	1242

26
84

GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA

G D A V A E I D A L Y D V Y L D V Q

1251 1260 1269 1278 1287 1296
GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC

E K W G L E D V M L M G D F N A G C

1305 1314 1323 1332 1341 1350
AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC

S Y V R P S Q W S S I R L W T S P T

1359 1368 1377 1386 1395 1404
TTC CAG TGG CTC ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT

F Q W L I P D S A D T T A T P T H C

1413 1422 1431 1440 1449 1458
GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CCA CCG GCC GTT GTT CCC

A Y D R I V V A G M L L R G A V V P

1467 1476 1485 1494 1503 1512
GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG

D S A L P F N P Q A A Y G L S D Q L

1521 1530 1539 1548 1557
GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'

A Q A I S D H Y P V E V M L K *

FIGURE 16

(A) pAS104

LOCUS PAS104.DNA 1560 bp mRNA PRI 06-MAR-1995
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS104)
 Position 924 G to A by ggg to gag
 Linker GR instead of GG (position 777)
 ACCESSION
 NID
 KEYWORDS DNase I.
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f
 MHDNASE1.dna)
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 AUTHORS Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis
 sputum
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
 MEDLINE 91067572
 BASE COUNT 346 a 468 c 434 g 312 t
 ORIGIN

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1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCACTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTCCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTIONTC CCCGAACCGG TGACGGTGTG GTGGAACCTA
541 GGCGCCCTGA CCAGCCGCCG GCACACCTTC CCGGCTGTCC TACAGTCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTCC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGCAGGCTG
781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC
841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA
901 GACAGCCACC TGACTIONCGT GCGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC
961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG
1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC
1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG
1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA
1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG
1261 GACGTCAATG TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCAGTGG
1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGTGAC
1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC
1441 CGAGGGGCGG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG
1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGTGA

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59
84

File : PAS104.DNA
 Range : 1 - 1560 Mode : Normal
 Codon Table : Universal

FIGURE 16(B)

5'	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC
	M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA
	S	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG
	V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT
	W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	P
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T
	ACA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG
	R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	CAC	TTT	GCC	TGG	TTT	GCT	TAC
	D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S
	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG
	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA
	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA
	C	A	L	T	S	C	V	H	T	F	P	A	V	L	Q	S	S	G

- 1 -

59/84

603	612	621	630	639	648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG					
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCG					
V E P K S C D K T H T C C V E C P P					
765	774	783	792	801	810
TGC CCA GCA CCT GAA GGC AGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT					
C P A P E G R L K I A A F N I Q T F					
819	828	837	846	855	864
GGG GAG ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTC					
G E T K M S N A T L V S Y I V Q I L					
873	882	891	900	909	918
AGC CGC TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC					
S R Y D I A L V Q E V R D S H L T A					
927	936	945	954	963	972
GTG GAG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC					
V E K L L D N L N Q D A P D T Y H Y					
981	990	999	1008	1017	1026
GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG					
V V S E P L G R N S Y K E R Y L F V					
1035	1044	1053	1062	1071	1080
TAC AGG CCT GAC CAG CTC TCT CCG CTG GAC AGC TAC TAC TAC GAT GAT GGC TGC					
Y R P D Q V S A V D S Y Y Y D D C C					
1089	1098	1107	1116	1125	1134
GAG CCC TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC					
E P C G N D T F N R E P A I V R F F					
1143	1152	1161	1170	1179	1188
TCC CGG TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG					
S R F T E V R E F A I V P L H A A P					
1197	1206	1215	1224	1233	1242

62/84

GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA

G D A V A E I D A L Y D V Y L D V Q

1251 1260 1269 1278 1287 1296
GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC

E K W G L E D V M L M G D F N A G C

1305 1314 1323 1332 1341 1350
AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC

S Y V R P S O W S S I R L W T S P T

1359 1368 1377 1386 1395 1404
TTC CAC TCG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT

F Q W L I P D S A D T T A T P T H C

1413 1422 1431 1440 1449 1458
GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC

A Y D R I V V A G M L L R G A V V P

1467 1476 1485 1494 1503 1512
GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG

D S A L P F N F Q A A Y G L S D Q L

1521 1530 1539 1548 1557
GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'

A Q A I S D H Y P V E V M L K +

FIGURE 17

(A) pAS105

LOCUS PAS105.DNA 1578 bp mRNA PRI 06-MAR-1995
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I with SV40 NLS(pAS105)
 ACCESSION
 NID
 KEYWORDS DNase I.
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f
 MHDNASE1.dna)
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 AUTHORS Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis
 sputum
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
 MEDLINE 91067672
 BASE COUNT 353 a 473 c 442 g 310 t
 ORIGIN

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1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCCG TGACGGTGTG GTGGAAGTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA CCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCUAG CAAGAACAAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAG GCGGGCTGAA GATCGCAGCC
781 TTCAACATCC AGACATTTGG GGAGACCAAG ATGTCCAATG CCACCCTCGT CAGCTACATT
841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTCAGAGA CAGCCACCTG
901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG CACCAGACAC CTATCACTAC
961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG
1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG
1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC
1141 AGGGAGTTTG CCATTGTTCC CCTGCATGCG GCCCCGGGGG ACGCAGTAGC CGAGATCGAC
1201 GCTCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG
1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCCT CCCAGTGGTC ATCCATCCGC
1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA
1381 CCCACGCACT GTGCCTATGA CAGGATCGTG GTTGCAGGGA TGCTGCTCCG AGGGGCCGTT
1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG
1501 GCCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGGGGGG CGGACCCAAA
1561 AAGAAGCGCA AGGTTTGA

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↳ NLS

62

84

File : PAS105.DNA
 Range : 1 - 1578 Mode : Normal
 Codon Table : Universal

FIGURE 17 (B)

5'	9	18	27	36	45	54
ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC						
M G W S C I I L F L V A T A T G V H						
63	72	81	90	99	108	
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA						
S Q V Q L V O S G A E V K K P G A S						
117	126	135	144	153	162	
GTG AAG GTG TCC TCC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG						
V K V S C K A S G Y T F S A Y W I E						
171	180	189	198	207	216	
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT						
W V R Q A P G K G L E W V G E I L P						
225	234	243	252	261	270	
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT						
G S N N S R Y N E K F K G R V T V T						
279	288	297	306	315	324	
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG						
R D T S T N T A Y M E L S S L R S E						
333	342	351	360	369	378	
GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC						
D T A V Y Y C A R S Y D F A W F A Y						
387	396	405	414	423	432	
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA CCC TCC ACC AAG GGC CCA TCG						
W G Q G T L V T V S S A S T K G P S						
441	450	459	468	477	486	
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG						
V F P L A P S S K S T S G G T A A L						
495	504	513	522	531	540	
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA						
G C L V K D Y F P E P V T V S W N S						
549	558	567	576	585	594	
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA						
G A L T S G V H T F P A V L Q S S G						

-1-

63/84

603	612	621	630	639	648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG					
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT					
V E P K S C D K T H T C P P C P A P					
765	774	783	792	801	810
GAA GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG					
E G G L K I A A F N I Q T F G E T K					
819	828	837	846	855	864
ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTC ACC CCC TAC GAC					
M S N A T L V S Y I V Q I L S R Y D					
873	882	891	900	909	918
ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG					
I A L V Q E V R D S H L T A V G K L					
927	936	945	954	963	972
CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG					
L D N L N Q D A P D T Y H Y V V S E					
981	990	999	1008	1017	1026
CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC					
P L G R N S Y K E R Y L F V Y R P D					
1035	1044	1053	1062	1071	1080
CAG CTC TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG					
Q V S A V D S Y Y Y D D G C E P C G					
1089	1098	1107	1116	1125	1134
AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTG AGG TTC TTC TCC CCG TTC ACA					
N D T F N R E P A I V R F F S R F T					
1143	1152	1161	1170	1179	1188
GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA					
E V R E F A I V P L H A A P G D A V					
1197	1206	1215	1224	1233	1242

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GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAC AAA TCC GGC

A E I D A L Y D V Y L D V Q E K W G

1251 1260 1269 1278 1287 1296
TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA

L E D V M L M G D F N A G C S Y V R

1305 1314 1323 1332 1341 1350
CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG

P S Q W S S I R L W T S P T F Q W L

1359 1368 1377 1386 1395 1404
ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG

I P D S A D T T A T P T H C A Y D R

1413 1422 1431 1440 1449 1458
ATC GTG GTT CCA CCC ATG CTG CTC CCA CCC GCC GTT GTT CCC GAC TCG GCT CTT

I V V A G M L L R G A V V P D S A L

1467 1476 1485 1494 1503 1512
CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC

P F N F Q A A Y G L S D Q L A Q A I

1521 1530 1539 1548 1557 1566
AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG

S D H Y P V E V M L K G G G P K K K

1575
CGC AAG GTT TGA 3'

R K V *

65
84

FIGURE 18

(A) pAS106

LOCUS PAS106.DNA 1596 bp mRNA PRI 06-MAR-1995
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I with SV40 NLS(pAS106)
 ACCESSION
 NID
 KEYWORDS DNase I.
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f
 MHDNASE1.dna)
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 AUTHORS Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis
 sputum
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
 MEDLINE 91067672
 BASE COUNT 355 a 475 c 452 g 314 t
 ORIGIN

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1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGAATACTTC CCCGAACCGG TGACGGGTGC GTGGAAGTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AAUGTGAATC ACAAGCCGAG CACACCCAGG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGGAGCGGC
781 GGGCTGAAGA TCGCAGCCTT CAACATCCAG ACATTTGGGG AGACCAAGAT GTCCAATGCC
841 ACCCTCGTCA GCTACATTGT GCAGATCCTG AGCCGCTACG ACATCGCCCT GGTCCAGGAG
901 GTCAGAGACA GCCACCTGAC TGCCGTGGGG AAGCTGCTGG ACAACCTCAA TCAGGACGCA
961 CCAGACACCT ATCACTACGT GGTCAGTGAG CCACTGGGAC GGAACAGCTA TAAGGAGCGC
1021 TACCTGTTTC TGTACAGGCC TGACCAGGTG TCTGCGGTGG ACAGCTACTA CTACGATGAT
1081 GGCTGCGAGC CCTGCGGGAA CGACACCTTC AACCAGAGAG CAGCCATTGT CAGGTTCTTC
1141 TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC ATTGTTCCCC TGCATGCGGC CCCGGGGGAC
1201 GCAGTAGCCG AGATCGACGC TCTCTATGAC GTCTACCTGG ATGTCCAAGA GAAATGGGGC
1261 TTGGAGGACG TCATGTTGAT GGGCGACTTC AATGCGGGCT GCAGCTATGT GAGACCTCC
1321 CAGTGGTCAT CCATCCGCCT GTGGACAAGC CCCACCTTCC AGTGGCTGAT CCCCAGACAGC
1381 GCTGACACCA CAGCTACACC CACGCACTGT GCCTATGACA GGATCGTGGT TGCAGGGATG
1441 CTGCTCCGAG GGGCCGTTGT TCCCGACTCG GCTCTTCCCT TTAACCTCCA GGCTGCCTAT
1501 GGCCTGAGTG ACCAACTGGC CCAAGCCATC AGTGACCACT ATCCAGTGA GGTGATGCTG
1561 AAGGGGGGCG GACCCAAAAA GAAGCGCAAG GTTTGA

```

//
 ↳ NLS

66
 84

File : PAS106.DNA
 Range : 1 - 1596 Mode : Normal
 Codon Table : Universal

FIGURE 18 (b)

```

      9      18      27      36      45      54
5' ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
   ---
   M  G  W  S  C  I  I  L  F  L  V  A  T  A  T  G  V  H

      63      72      81      90      99      108
TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
   ---
   S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S

      117     126     135     144     153     162
GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
   ---
   V  K  V  S  C  K  A  S  G  Y  T  F  S  A  Y  W  I  E

      171     180     189     198     207     216
TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
   ---
   W  V  R  Q  A  P  G  K  G  L  E  W  V  G  E  I  L  P

      225     234     243     252     261     270
GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
   ---
   G  S  N  N  S  R  Y  N  E  K  F  K  G  R  V  T  V  T

      279     288     297     306     315     324
AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
   ---
   R  D  T  S  T  N  T  A  Y  M  E  L  S  S  L  R  S  E

      333     342     351     360     369     378
GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
   ---
   D  T  A  V  Y  Y  C  A  R  S  Y  D  F  A  W  F  A  Y

      387     396     405     414     423     432
TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA CCC TCC ACC AAG GGC CCA TCC
   ---
   W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G  P  S

      441     450     459     468     477     486
GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
   ---
   V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L

      495     504     513     522     531     540
GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
   ---
   G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S

      549     558     567     576     585     594
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
   ---
   G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G

```

- 1 -

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CCC CCG CCC CAC GCA GTA CCC CAC ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT

A P G D A V A E I D A L Y D V Y L D

1251 1260 1269 1278 1287 1296
GTC CAA GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG

V Q E K W G L E D V M L M G D F N A

1305 1314 1323 1332 1341 1350
GGC TGC AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC

G C S Y V R P S Q W S S I R L W T S

1359 1368 1377 1386 1395 1404
CCC ACC TTC CAG TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG

P T F Q W L I P D S A D T T A T P T

1413 1422 1431 1440 1449 1458
CAC TGT GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT

H C A Y D R I V V A G M L L R G A V

1467 1476 1485 1494 1503 1512
GTT CCC GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC

V P D S A L P F N F Q A A Y G L S D

1521 1530 1539 1549 1557 1566
CAA CTG GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG

Q L A Q A I S D H Y P V E V M L K G

1575 1584 1593
GGC GGA CCC AAA AAG AAG CGC AAG GTT TGA 3'

G G P K K K R K V *

603	612	621	630	639	648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG					
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCC					
V E P K S C D K T H T C C V E C P P					
765	774	783	792	801	810
TGC CCA GCA CCT GAA GGG AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG					
C P A P E G S G G L K I A A F N I Q					
819	828	837	846	855	864
ACA TTT GGG GAG ACC AAG ATG TCC AAT GCC ACC CTC CTC ACC TAC ATT GTG CAG					
T F G E T K M S N A T L V S Y I V Q					
873	882	891	900	909	918
ATC CTG AGC CGC TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG					
I L S R Y D I A L V Q E V R D S H L					
927	936	945	954	963	972
ACT GCC GTG GGG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT					
T A V G K L L D N L N Q D A P D T Y					
981	990	999	1008	1017	1026
CAC TAC GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG					
H Y V V S E P L G R N S Y K E R Y L					
1035	1044	1053	1062	1071	1080
TTC GTG TAC AGG CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT					
F V Y R P D Q V S A V D S Y Y Y D D					
1089	1098	1107	1116	1125	1134
GGC TGC GAG CCC TGC GGC AAC CAC ACC TTC AAC CGA CAG CCA CCC ATT GTC AGG					
G C E P C G N D T F N R E P A I V R					
1143	1152	1161	1170	1179	1188
TTC TTC TCC CGG TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG					
F F S R F T E V R E F A I V P L H A					
1197	1206	1215	1224	1233	1242

62
84

FIGURE 19

(A) pAS107

LOCUS PAS107.DNA 1590 bp mRNA PRI 06-MAR-1995
 DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I with SV40 NLS (pAS107)
 ACCESSION
 NID
 KEYWORDS DNase I.
 SOURCE DNase I sequence is from assembled oligos (thus modified c/f
 MHDNASE1.dna)
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 AUTHORS Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
 TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis
 sputum
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
 MEDLINE 91067672
 BASE COUNT 354 a 474 c 448 g 314 t
 ORIGIN

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1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCAG
61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAAGTGC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GGCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGGTGTC GTGGAAGTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGCGGGGCTG
781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC
841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTGAGA
901 GACAGCCACC TGACTGCCGT GGGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC
961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG
1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC
1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG
1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA
1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG
1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG
1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC
1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC
1441 CGAGGGGCGG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG
1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGGGG
1561 GGCGGACCCA AAAAGAAGCG CAAGGTTTGA

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↳ NLS

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File : FAS107.DNA
Range : 1 - 1590 Mode : Normal
Codon Table : Universal

FIGURE 19 (B)

5'	9	18	27	36	45	54											
ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC
M	G	W	S	C	I	I	L	F	L	V	A	T	A	T	G	V	H
63	72	81	90	99	108												
TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA
S	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S
117	126	135	144	153	162												
GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG
V	K	V	S	C	K	A	S	G	Y	T	F	S	A	Y	W	I	E
171	180	189	198	207	216												
TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT
W	V	R	Q	A	P	G	K	G	L	E	W	V	G	E	I	L	P
225	234	243	252	261	270												
GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT
G	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T
279	288	297	306	315	324												
AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG
R	D	T	S	T	N	T	A	Y	M	E	L	S	S	L	R	S	E
333	342	351	360	369	378												
GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC
D	T	A	V	Y	Y	C	A	R	S	Y	D	F	A	W	F	A	Y
387	396	405	414	423	432												
TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	CCC	TCC	ACC	AAG	GGC	CCA	TCG
W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S
441	450	459	468	477	486												
GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG
V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L
495	504	513	522	531	540												
GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA
G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S
549	558	567	576	585	594												
GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA
G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G

-1-

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603	612	621	630	639	648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG					
L Y S L S S V V T V P S S S L G T Q					
657	666	675	684	693	702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA					
T Y I C N V N H K P S N T K V D K K					
711	720	729	738	747	756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCG					
V E P K S C D K T H T C C V E C P P					
765	774	783	792	801	810
TGC CCA GCA CCT GAA GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT					
C P A P E G G L K I A A F N I Q T F					
819	828	837	846	855	864
GGG GAG ACC AAG ATG TCC AAT CCC ACC CTC GTC AGC TAC ATT CTG CAG ATC CTG					
G E T K M S N A T L V S Y I V Q I L					
873	882	891	900	909	918
AGC CGC TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC					
S R Y D I A L V Q E V R D S H L T A					
927	936	945	954	963	972
GTG GGG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC					
V G K L L D N L N Q D A P D T Y H Y					
981	990	999	1008	1017	1026
GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG					
V V S E P L G R N S Y K E R Y L F V					
1035	1044	1053	1062	1071	1080
TAC AGG CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC					
Y R P D Q V S A V D S Y Y Y D D G C					
1089	1098	1107	1116	1125	1134
GAC CCC TCC GGG AAC GAC ACC TTC AAC CGA GAG CCA CCC ATT CTC AGG TTC TTC					
E P C G N D T F N R E P A I V R F F					
1143	1152	1161	1170	1179	1188
TCC CGG TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG					
S R F T E V R E F A I V P L H A A P					
1197	1206	1215	1224	1233	1242

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GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT CTC CAA

G D A V A E I D A L Y D V Y L D V Q

1251 1260 1269 1278 1287 1296
GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC

E K W G L E D V M L M G D F N A G C

1305 1314 1323 1332 1341 1350
AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC

S Y V R P S Q W S S I R L W T S P T

1359 1368 1377 1386 1395 1404
TTC CAG TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT

F Q W L I P D S A D T T A T P T H C

1413 1422 1431 1440 1449 1458
GCC TAT GAC AGC ATC CTC GTT GCA GGG ATG CTC CTC CGA GGG GCC GTT GTT CCC

A Y D R I V V A G M L L R C A V V P

1467 1476 1485 1494 1503 1512
GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG

D S A L P F N F Q A A Y G L S D Q L

1521 1530 1539 1548 1557 1566
GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA

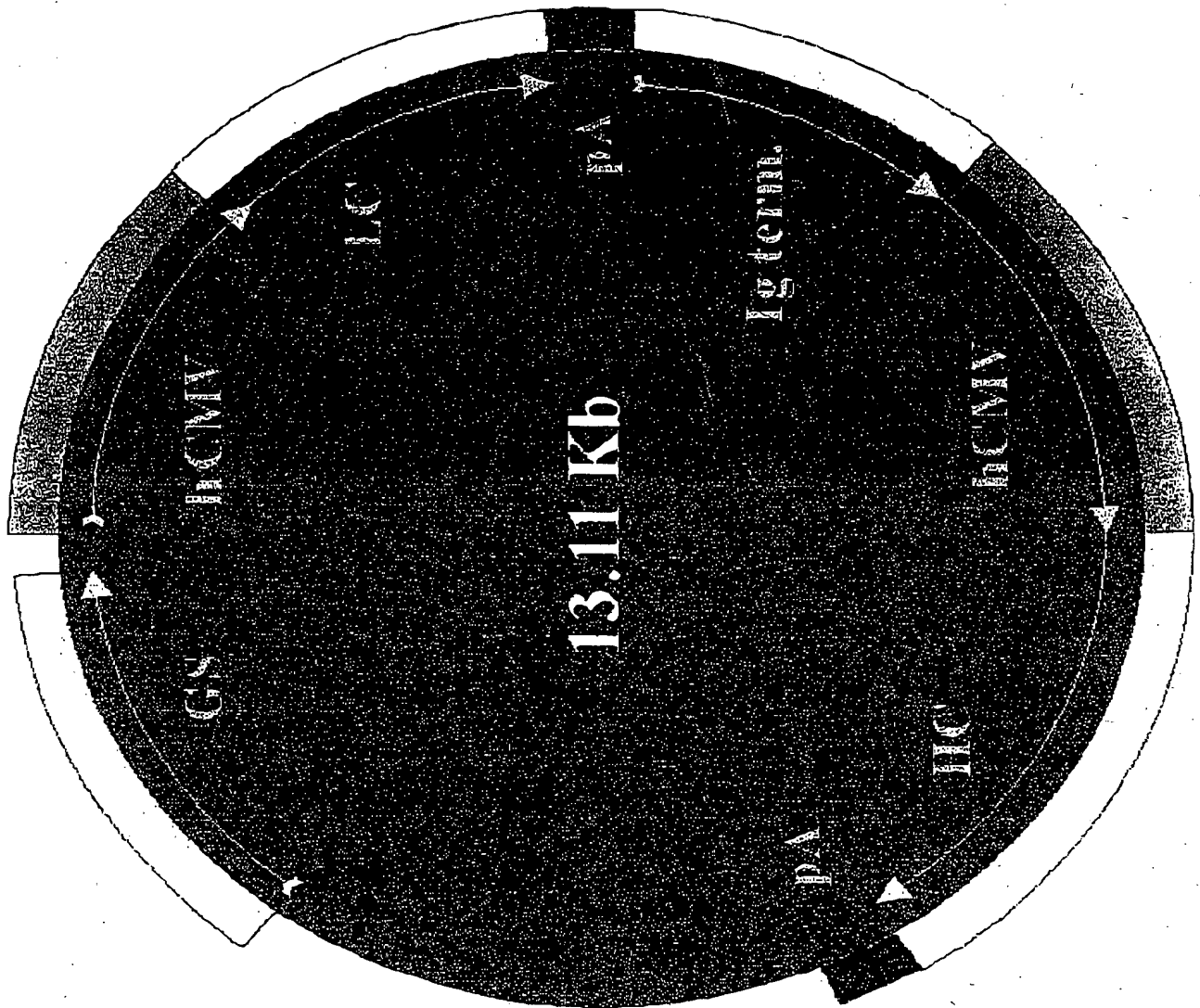
A Q A I S D H Y P V E V M L K G G G

1575 1584
CCC AAA AAG AAG CGC AAG GTT TGA 3'

P K K K R K V *

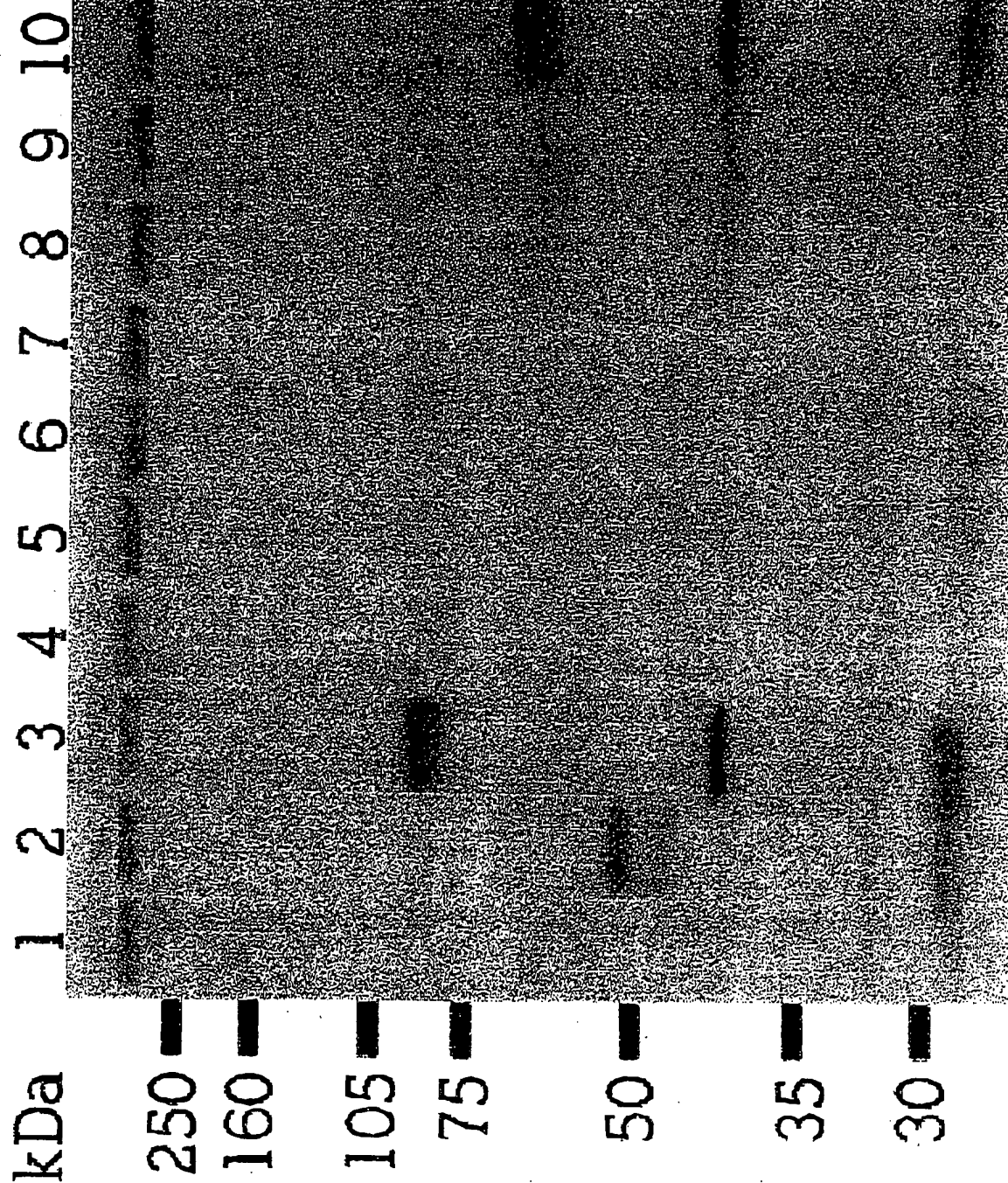
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Fig 20 Mammalian expression of humanised HMFG1-DNase constructs



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/84

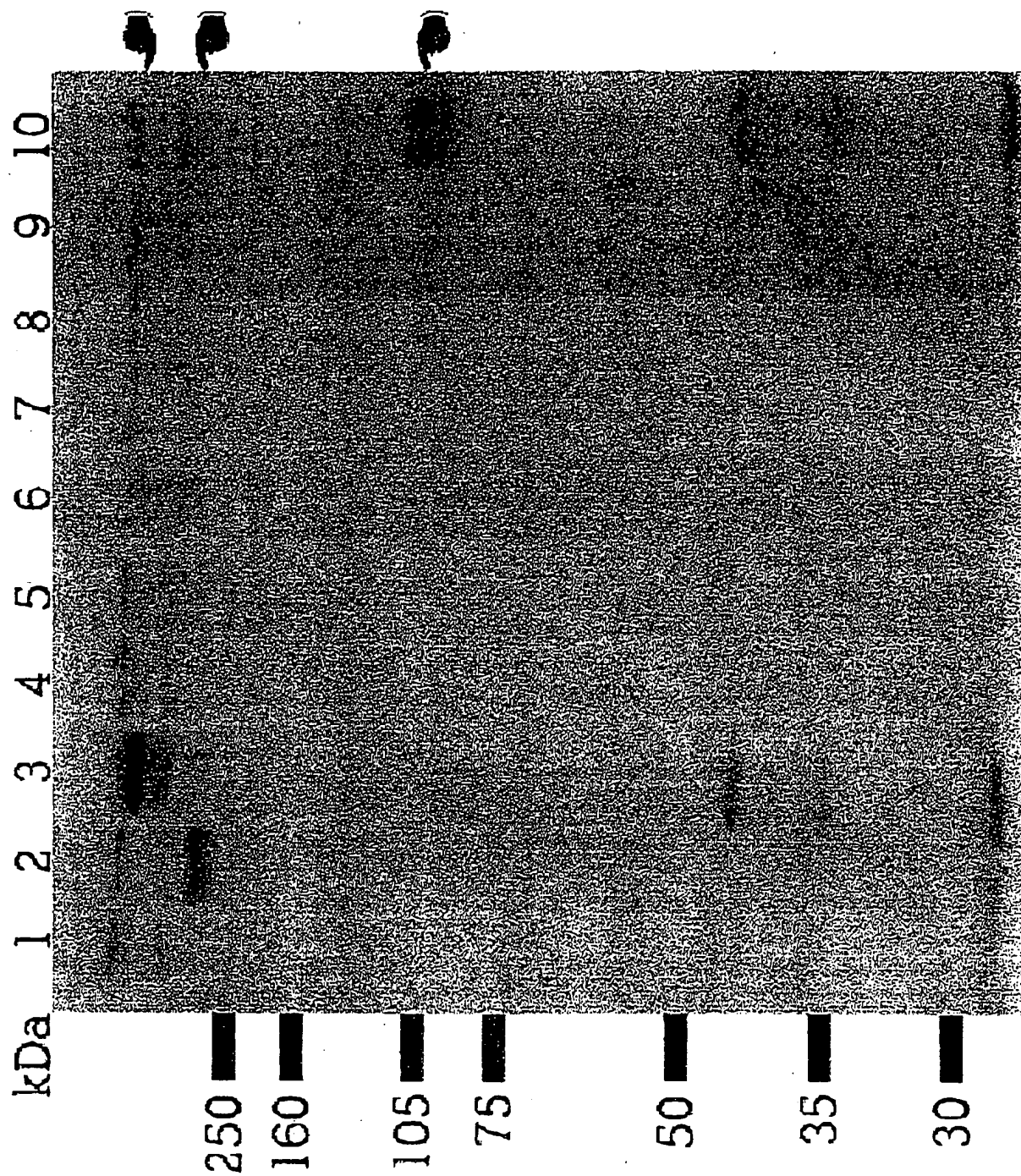
Fig 2(a) Immuno-precipitation of metabolically labelled transient transfectants



8% SDS-PAGE reducing gel

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Fig 21
(b)
Immuno-precipitation of metabolically labelled transient transfectants



8% SDS-PAGE non-reducing gel

Fig 21 Immuno-precipitation of metabolically labelled transient transfectants

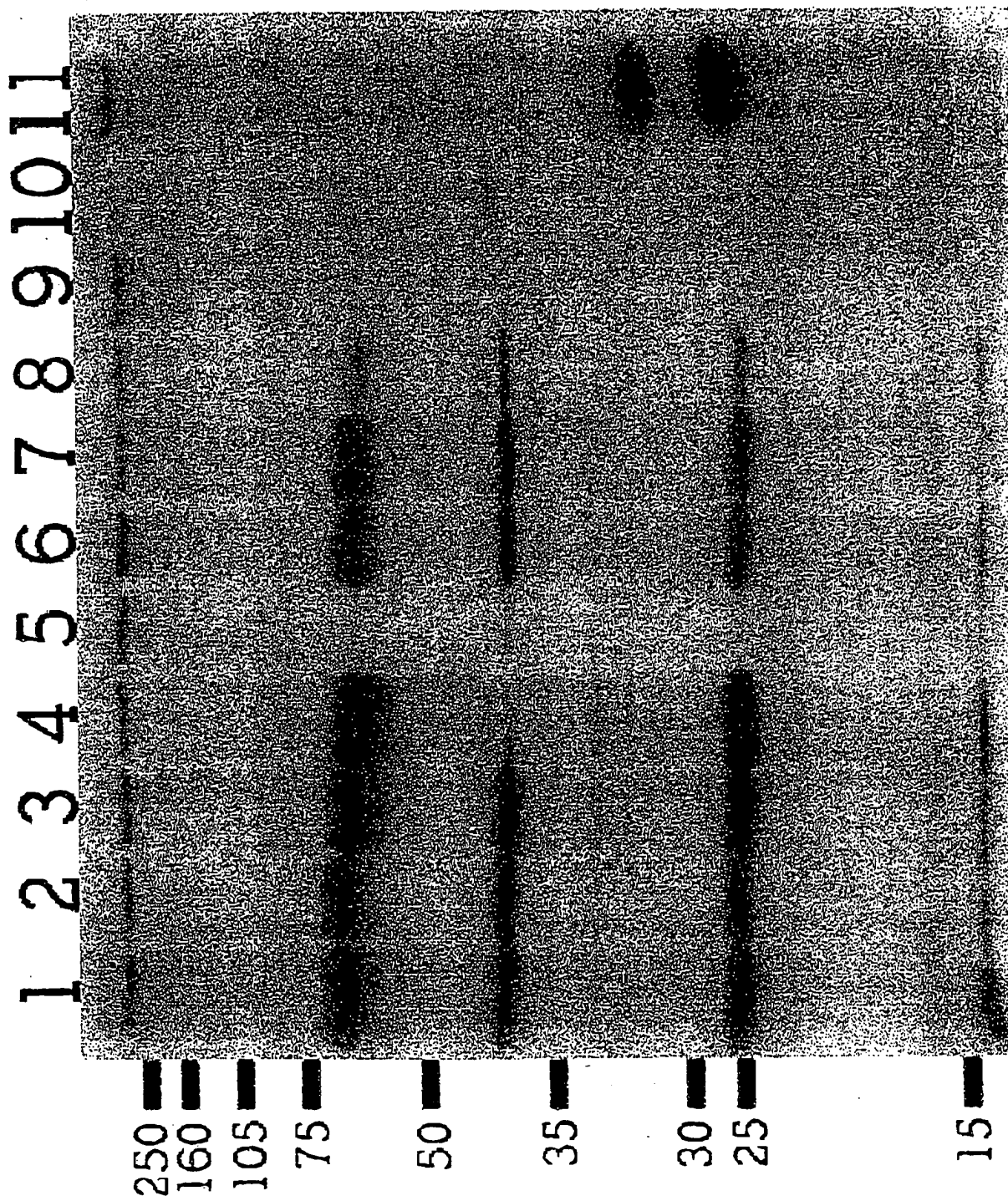


Fig 21

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Fig 21 Immuno-precipitation of metabolically labelled transient transfectants

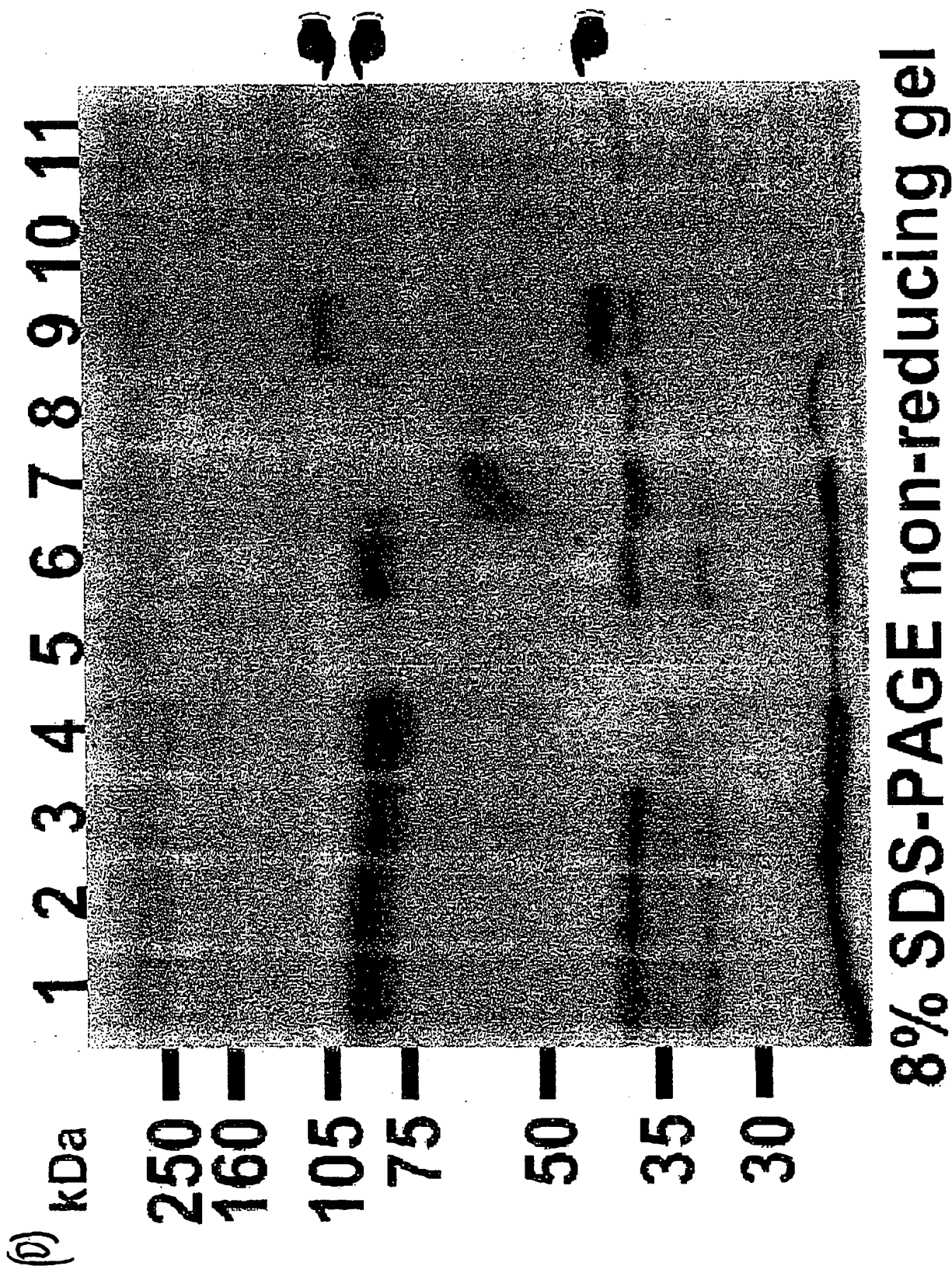


Fig 21

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PDTRP binding assay standard curve
(5' development)

Corrected bovine DNase I standard curves
at various time points

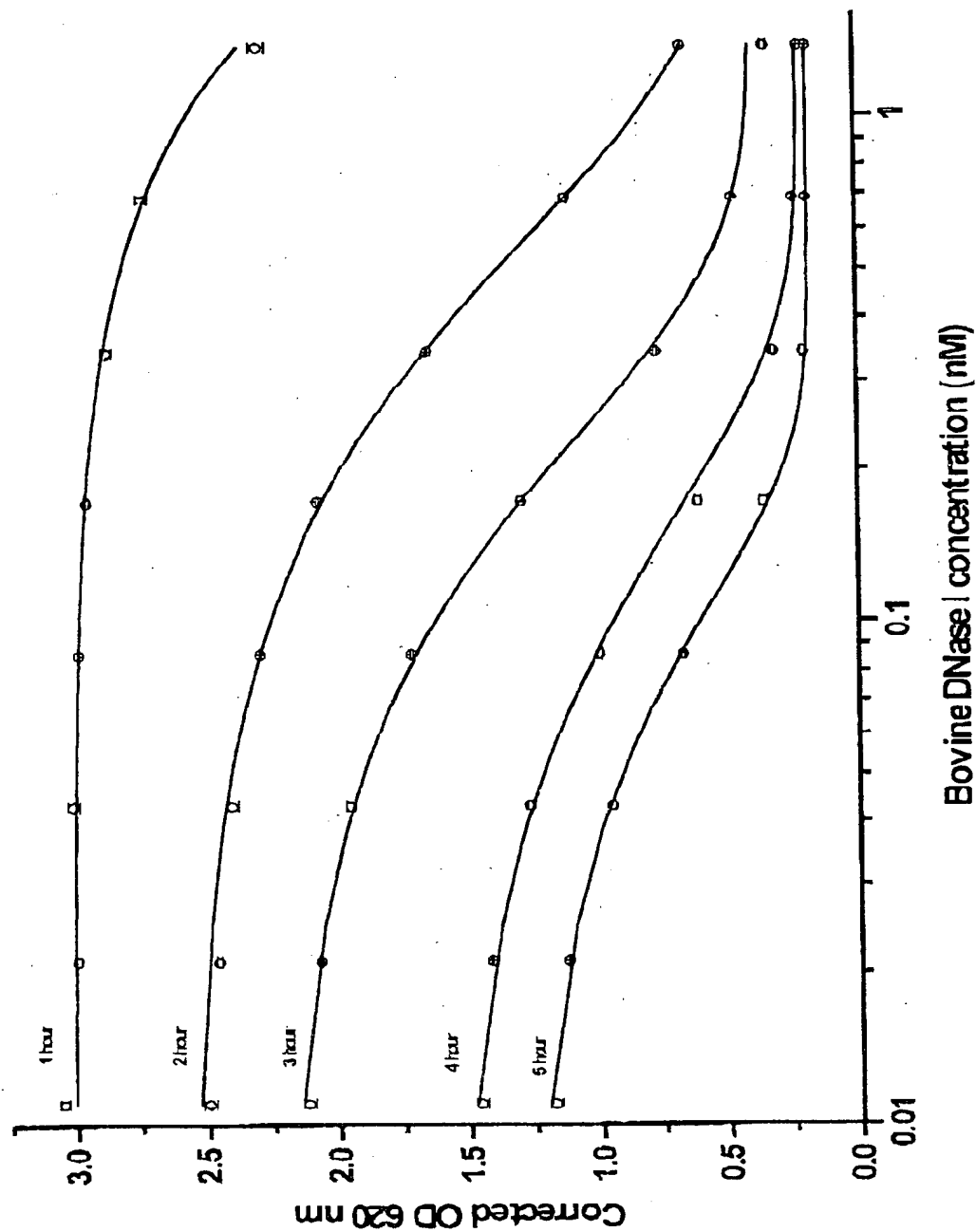


FIG 23

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FIG 24

Corrected DNase I activity in transiently expressed
humanised human HMFG1-human DNase I constructs

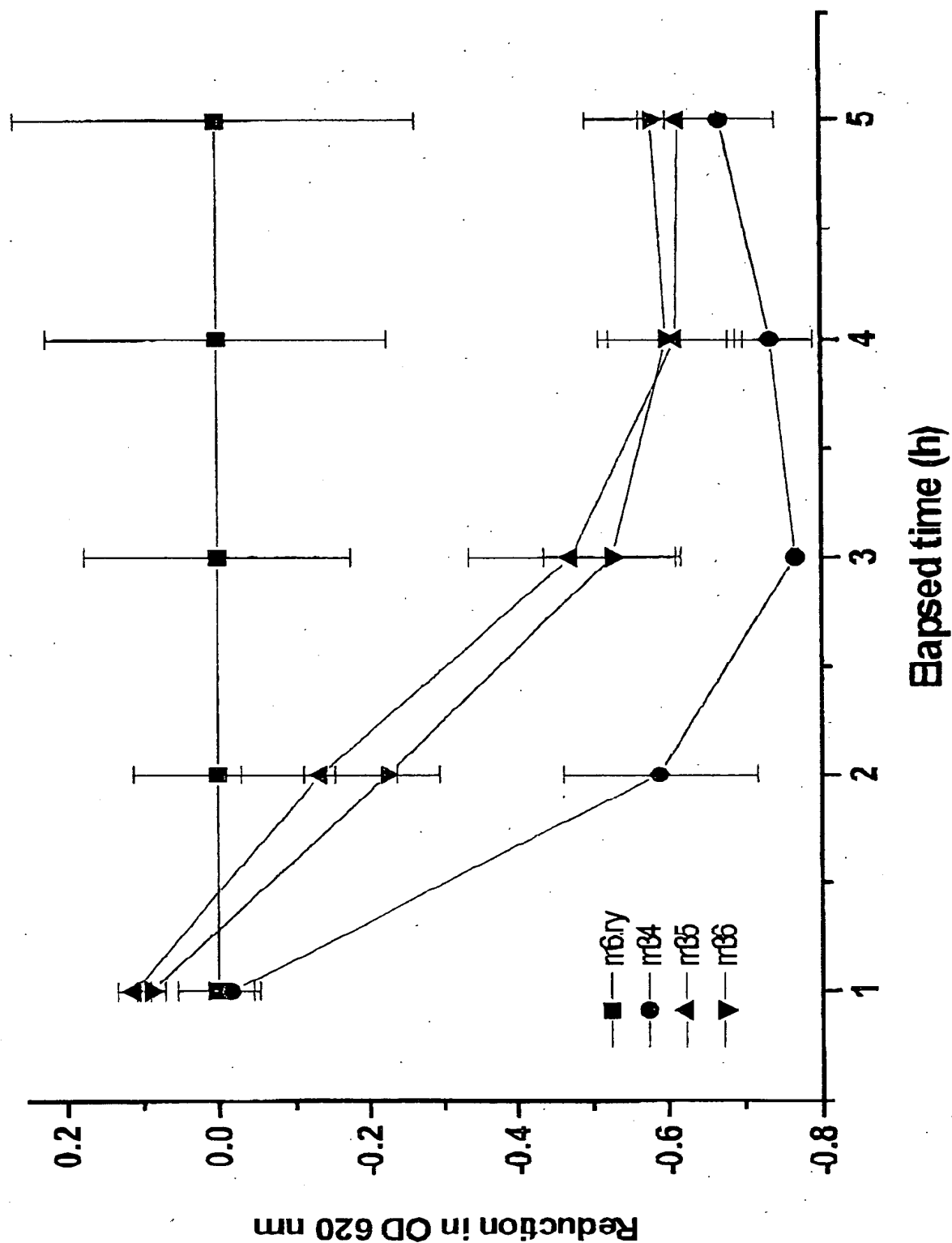


FIG 28

Corrected DNase I activity in transiently expressed
humanised HMFG1 F(ab')₂-human DNase I fusions

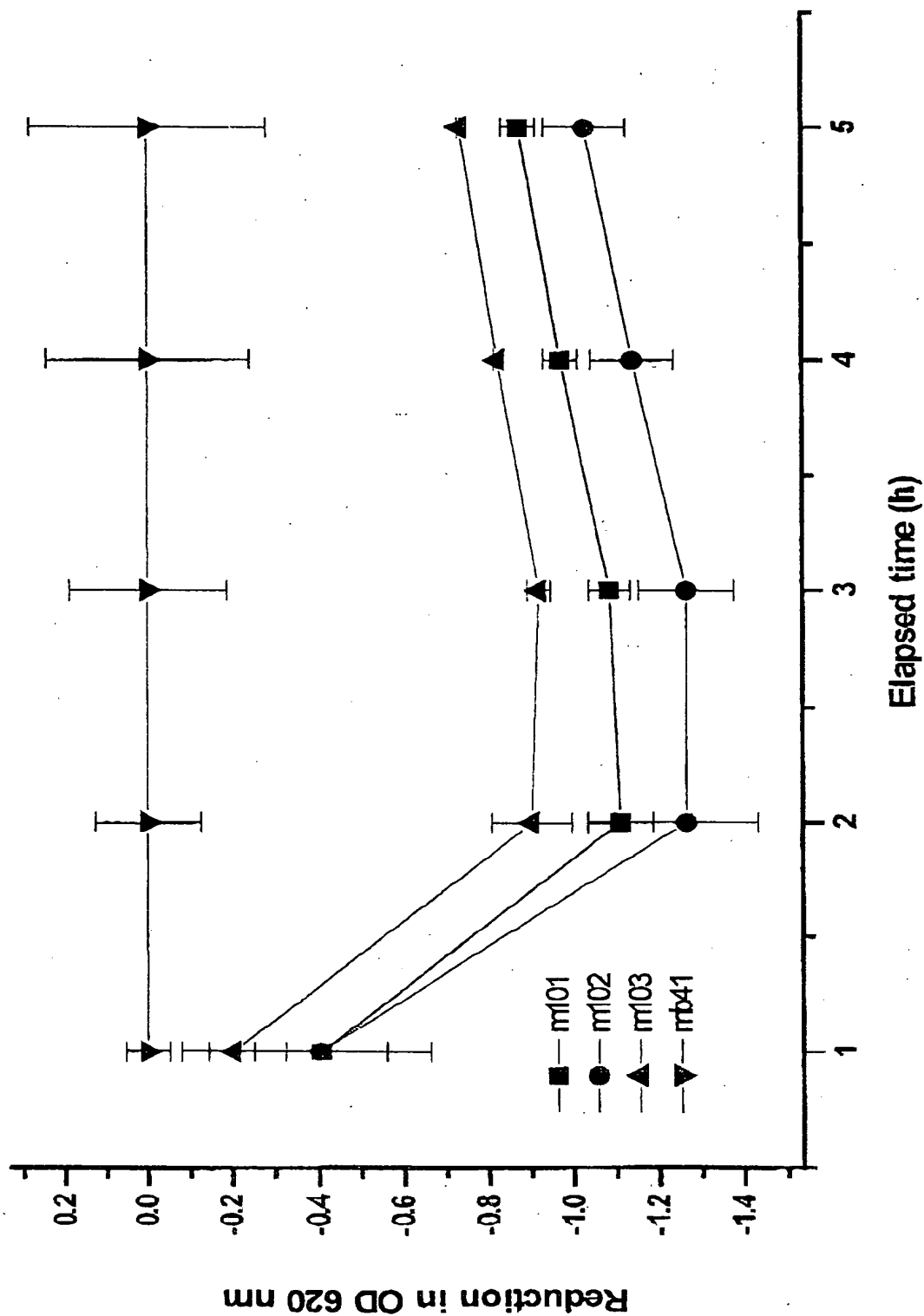
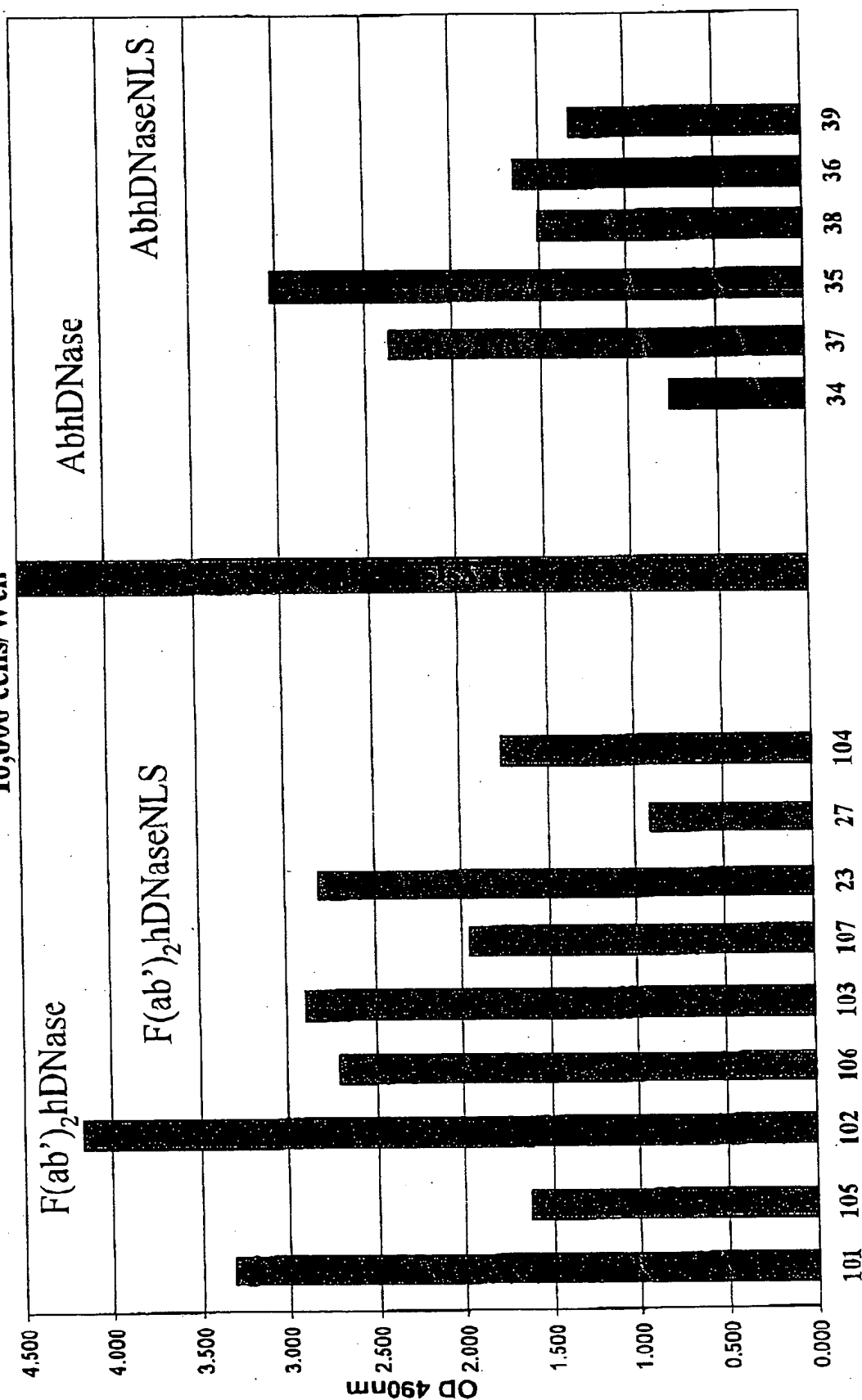
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FIG. 26

Cytotoxicity Assay

10,000 cells/Well



0.097 µg/ml of each construct

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MCF7 cells killed after 1h incubation with 1.35 ng of sample

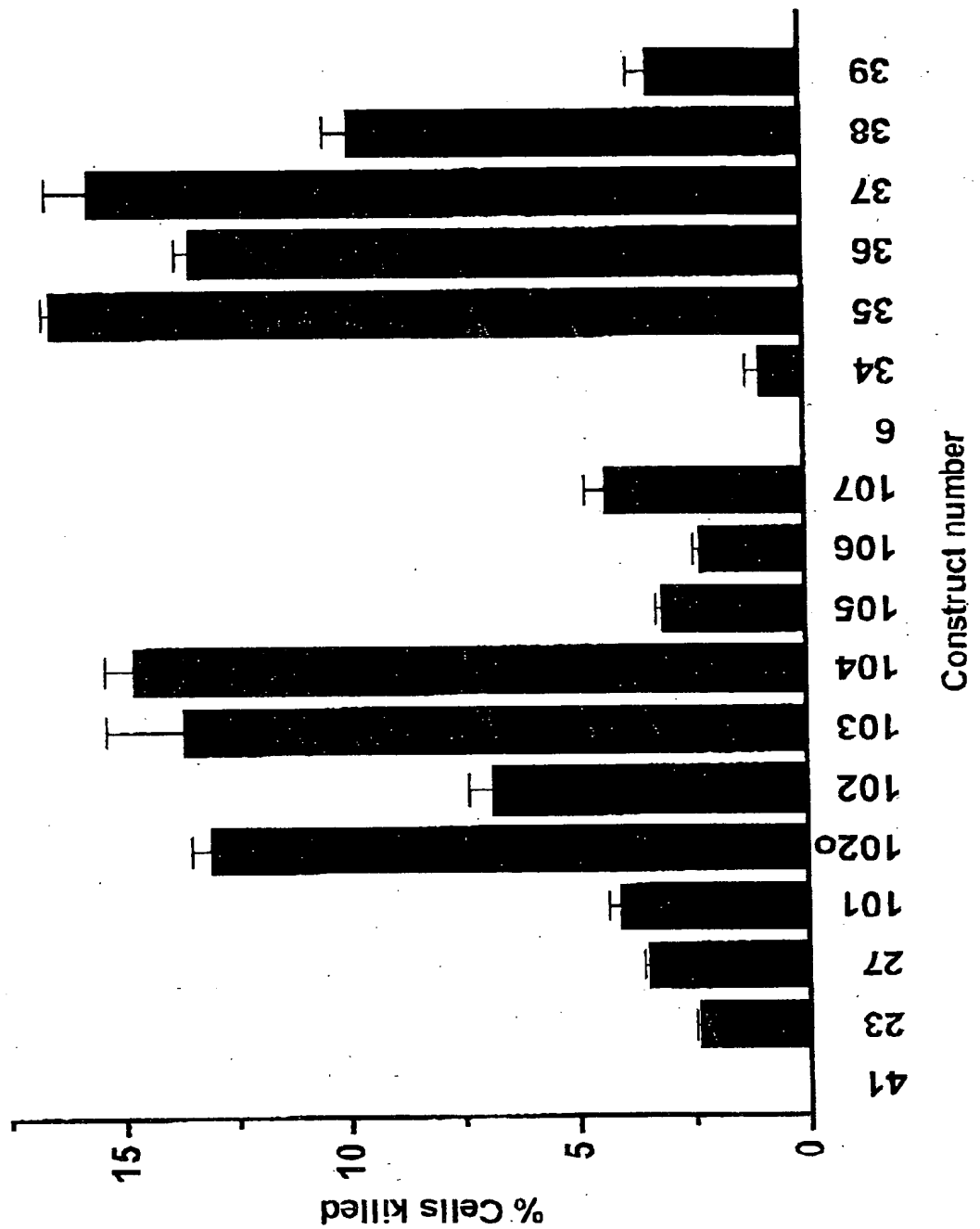


FIG 27

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